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# Ecological Assessment of Hazardous Waste Sites
# A Field and Laboratory Reference

Kilkelly Environmental Associates, Inc., Raleigh, NC

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Abstract: The document discusses the scientific basis for assessing adverse ecological effects at hazardous waste sites (HWSs), and presents methods for evaluating the on-site and off-site ecological effects of HWSs. The methods are intended for implementation in the early phases of the HWS evaluation process and should be used as integral parts of HWS evaluations. The document is intended for use by administrative and scientific personnel with a strong background in the environmental sciences, including laboratory and field procedures, and environmental assessment strategies.

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# ECOLOGICAL ASSESSMENT OF HAZARDOUS WASTE SITES: A FIELD AND LABORATORY REFERENCE

Edited By

William Warren-Hicks[1]

Benjamin R. Parkhurst[2]

Samuel S. Baker, Jr.[1]

[1]Kilkelly Environmental Associates
Highway 70 West - The Water Garden
Raleigh, NC 27622

[2]Western Aquatics, Inc.
P.O. Box 546
203 Grand Avenue
Laramie, WY 82070

# DISCLAIMER

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# EXECUTIVE SUMMARY

This report is a field and laboratory reference document that provides guidance on designing, implementing, and interpreting ecological assessments of hazardous waste sites. It is comprised of nine chapters that address the following: (1) the definition of an ecological assessment, (2) evaluation and selection of appropriate ecological endpoints, (3) basic strategies and approaches to ecological assessments, (4) considerations in field sampling design, (5) the role of quality assurance and quality control, (6) recommended aquatic and terrestrial toxicity tests, (7) recommended biomarkers, (8) recommended aquatic and terrestrial field survey methods, and (9) considerations in data analysis and interpretation. The report discusses the scientific basis for assessing adverse ecological effects at a hazardous waste site and presents methods for evaluating the ecological effects associated with toxic hazardous waste site chemicals.

The methods are intended for implementation in the early phases of the hazardous waste site evaluation process and should be used as integral parts of hazardous waste site studies. The methods presented in this document can be implemented within a time frame of 12 to 18 months and, in some cases, the analyses can be completed in a matter of days.

The methods presented in this document are not required by regulation. However, they provide a reasonable basis for assessing the adverse ecological effects associated with hazardous waste sites.

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# CHAPTER 1

# INTRODUCTION

## 1.1 PURPOSE

This document has the following purposes: (1) to discuss the scientific basis for assessing adverse ecological effects at hazardous waste sites (HWSs), and (2) to present methods for evaluating the on-site and off-site ecological effects of HWSs. The methods are intended for implementation in the early phases of the HWS evaluation process and should be used as integral parts of HWS evaluations. This document is intended for use by administrative and scientific personnel with a strong background in the environmental sciences, including laboratory and field procedures, and environmental assessment strategies.

## 1.2 BACKGROUND

A high priority of the U.S. EPA is to identify, characterize, and cleanup HWSs. These activities are regulated by the Comprehensive Environmental Response Compensation and Liability Act (CERCLA), as amended by the Superfund Amendment and Reauthorization Act of 1986 (SARA). Both CERCLA and SARA address the toxic effects of hazardous wastes to aquatic and terrestrial organisms; consequently, environmental toxicity is one of the principal characteristics used to identify and characterize HWSs. Many of the methods presented in this document have been adapted from toxicity-based approaches to environmental assessment. The toxicity-based approach was developed for measuring and assisting in the regulation of toxic complex effluents discharged to surface waters (U.S. EPA 1985). It has also been used to identify and characterize toxic wastes under regulations enforced by the Resource Conservation and Recovery Act (RCRA) of 1976 as amended (Millemann and Parkhurst 1980). While site-specific characteristics may influence the assessment strategy at a HWS, the potential list of "appropriate, relevant, and applicable regulations" (ARARs) in force under CERCLA and SARA could provide a basis for selecting methodologies applicable to a given site, particularly if mandated through legislation (e.g., Clean Water Act, Endangered Species Act and the Safe Drinking Water Act).

Three types of information are needed to establish a firm, causal relationship between toxic wastes and ecological effects. First, chemical analyses of the appropriate media are necessary to establish the presence, concentrations, and variabilities of specific toxic chemicals. Second, ecological surveys are necessary to establish that adverse ecological effects have occurred. And finally, toxicity tests are necessary to establish a link between the adverse ecological effects and the toxicity of the wastes. Without all three types of data, other potential causes of the observed effects unrelated to the toxic effects of hazardous wastes, such as habitat alterations and natural variability, cannot be eliminated. For the following reasons, confidence in cleanup and monitoring decisions is greatly enhanced when based on a combination of chemical, ecological, and toxicological data:

- Ecological and toxicological data can be used to assess the aggregate toxicity of all toxic constituents at an HWS.
- The bioavailability of toxic chemicals is measured with ecological and toxicological assessments, but not with chemical analyses; therefore, the use of chemical data alone may over or underestimate the toxicities of single chemicals.
- Ecological and toxicological assessments link chemical-specific toxicity with measured biological responses, thereby providing a realistic assessment of environmental effects.
- Ecological and toxicological assessments provide information on the magnitude and variation of toxic effects, which may be useful in cleanup and monitoring strategies.

## 1.3 DEFINITION OF AN ECOLOGICAL ASSESSMENT

The objective of an ecological assessment is to quantify the ecological effects occurring at an HWS. In this document, ecological effects refer principally to population- and community-level effects on terrestrial and aquatic biota and biological processes. The magnitude and extent of ecological effects are measured based on a select set of ecological endpoints that are considered reasonable indices of the status of biological populations and communities on and near HWSs.

The expected outputs from an ecological assessment include the following:

- A basic inventory of the current status of selected components of the biological community in the area.
- An estimate of the current level of ecological effects associated with the HWS based on the selected subset of ecological endpoints.
- An estimate of the magnitude and variation of toxic effects.
- To the degree possible, identification of the extent to which these effects have resulted specifically from the presence of hazardous and toxic chemicals, as opposed to other associated effects such as habitat disruption.

Outputs not expected from an ecological assessment include the following:

- Predictions of future ecological effects at the HWS.
- An assessment of risk, although the data generated will be a useful component of an environmental risk analysis.
- Analyses specific to optimizing the design of remedial actions, assessing potential effects on human health, and evaluating the fate and transport of hazardous wastes. However, the data generated from an ecological assessment may contribute significantly to such analyses.
- Comprehensive ecological studies or research investigations. Ecological assessments of HWSs will focus on selected ecological endpoints.

Ecological assessments are a single component of an HWS evaluation. Other studies at the site include chemical analyses to establish the occurrence and distribution of potentially hazardous substances in the environment, models that predict the fate and transport of chemical substances at the site, and assessments of the threat to human health. The assessment methods presented in this section should be integrated with these analyses as part of the HWS evaluation process.

## 1.4 CRITERIA FOR METHODS SELECTION AND PRESENTATION

Some of the methods presented in this document are well developed, widely accepted procedures while others are less standard. This discrepancy is due, in part, to a differing amount of scientific research in methods development within specific environmental areas. For example, methods of toxicity assessment in freshwater systems are well developed while methods of toxicity testing in terrestrial systems are less well developed. To reflect the present state-of-the-science, the laboratory and field methods presented in this document are categorized into two classes, I and II. Class I methods represent standardized off-the-shelf methods, i.e., ones that have been extensively researched and validated for use in environmental assessments. In most cases, a large body of existing information is available documenting the ability of the test results to confirm the existence of adverse ecological effects. Class II tests represent test methods that are still under development, but which may be applicable to specific environmental situations at an HWS. Class II methods have not undergone the amount of standardization and validation associated with Class I methods. However, Class II methods should not be considered inferior methods. They may be the procedures of choice for site-specific evaluations or may be the only methods available at this time. Within this document, the advantages and disadvantages of Class I and Class II methods are presented, where appropriate.

Step-by-step details are not included for conducting the methods presented in this document. Rather, specific tests and procedures are recommended, and selected references are provided. The reader should consult the reference(s) for specific, detailed guidance on implementing a desired procedure. In addition, information useful for selecting a specific method, the expected outputs from the method, and the strengths and weaknesses of the method are discussed, where appropriate.

The methods presented in this document can be implemented within a time frame of 12 to 18 months. Methods requiring longer periods of time were not included. Given that environmental conditions vary greatly among sites, the selected methods are sufficiently flexible to permit implementation at most sites.

This document should be used in conjunction with the Superfund Environmental Evaluation Manual, currently under development by the U.S. EPA Office of Solid Waste and Emergency Response (OSWER). The reader is directed to the OSWER document for further guidance on the role of ecological assessment within the Superfund program. Additionally, other federal agencies have developed summary documents which may be relevant to HWS evaluation on a site-specific basis (U.S. FWS 1987).

## 1.5 ORGANIZATION OF THE DOCUMENT

This document is a field and laboratory reference document that provides guidance on designing, implementing, and interpreting an ecological assessment. It is comprised of nine chapters that address the following subjects: (1) the introduction, (2) evaluation and selection of appropriate ecological endpoints, (3) basic strategies and approaches to ecological assessments, (4) considerations in field sampling design, (5) the role of quality assurance and quality control in HWS evaluations, (6) recommended aquatic and terrestrial toxicity tests, (7) recommended biomarkers, (8) recommended aquatic and terrestrial field survey methods, and (9) considerations in data analysis and interpretation.

Each chapter in this document presents a discussion of issues and methods related to designing, implementing, and interpreting ecological assessments of hazardous waste sites. The authors of each of these chapters presented their papers at a workshop held in Seattle, WA on July 25-27, 1988. Workshop participants are presented in Appendix A. During the workshop, the material contained in this document was presented and discussed, and many of the comments received during the workshop have been incorporated. As new information on ecological assessment becomes available, new techniques undoubtedly will be developed. The methods and recommendation presented in this document will, as a consequence, be revised.

## 1.6 REFERENCES

Millemann, R.E., and B.R. Parkhurst. 1980. Comparative toxicity of solid waste leachates to Daphnia magna. Environ. Internet. 4:255-260.

Public Law 94-580. 1976. Resource Conservation and Recovery Act (RCRA), as amended.

Public Law 96-510. 1980. Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), as amended.

Public Law 99-499. 1986. Superfund Amendment and Reauthorization Act (SARA), as amended.

U.S. Department of Interior. 1987. Type B Technical Information Document. Injury to Fish and Wildlife Species. CERCLA Project 301. Washington, DC.

U.S. Environmental Protection Agency. 1985. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. EPA/600/4-85/014, Environmental Monitoring and Support Laboratory, Cincinnati, OH. 162 pp.

U.S. Environmental Protection Agency. In preparation. Superfund Environmental Evaluation Manual. Office of Solid Waste and Emergency (OSWER), Washington, DC.

# CHAPTER 2

# ECOLOGICAL ENDPOINTS

By

**Glenn W. Suter II, Environmental Sciences Division,
Oak Ridge National Laboratory, Oak Ridge, TN.**

## 2.1 INTRODUCTION

The purpose of ecological assessment of hazardous waste sites is to provide input to the decision making processes associated with a broad range of applications including site prioritization, waste characterization, site characterization, cleanup or remediation assessment, and site monitoring. The results of the ecological assessment that constitute the input to the decision making processes are descriptions of the relationship of pollutants to ecological endpoints. If the ecological endpoints are not compelling, they will not contribute to the decision. This chapter describes two different types of endpoints, presents criteria for judging endpoints, presents classes of endpoints that are potentially useful in assessments of waste sites, judges them by the criteria, and discusses how the nature of the assessment problem affects endpoint choice.

## 2.2 TYPES OF ENDPOINTS

Some confusion may occur in the practice of environmental assessment because the term endpoint has been used to describe two related but distinct concepts. To avoid this confusion, the following paragraphs distinguish assessment endpoints from measurement endpoints.

Assessment endpoints are formal expressions of the actual environmental values that are to be protected. Ecological assessments, as defined in this document, are concerned with describing the existing effects of a hazardous waste site on the environment. Therefore, the assessment endpoints are environmental characteristics, which, if they were found to be significantly affected, would indicate a need for remediation.

Assessment endpoints must be valued, but they are not ultimate values. Rather, they are the highest values that can be objectively assessed. Ultimate values fall in the domain of risk management, where ecological and human health assessment results are considered along with political, legal, economic, and ethical values to arrive at a plan for remediation.

A measurement endpoint is a quantitative expression of an observed or measured effect of the hazard; it is a measurable environmental characteristic that is related to the valued characteristic chosen as an assessment endpoint. In some cases, the measurement endpoint may be the same as the assessment endpoint. If the assessment endpoint for a waste site is decreased abundance of green sunfish in a stream adjoining the site, then abundance of the sunfish can be measured and related to abundance in reference sites. Because some potential assessment endpoints are not observable or measurable, and because assessments are often limited to using available of standard data, measurement endpoints are often surrogates for assessment endpoints. For example if the assessment endpoint is reduced production of green sunfish in the stream due to toxic effects of the leachate, productivity can not be measured in the time allotted to a typical field study and toxic effects can not be reliable separated in the field from other effects on productivity. In that case, toxicity test endpoints are appropriate but they are likely to be standard EPA test endpoints such as a fathead minnow LC50 for the leachate. When the measurement endpoint is not the same as the assessment endpoint, then some model must express the relationship between the two. It may be as simple as: a fish is a fish and so fathead minnows can simulate green sunfish, and population production would probably be affected at the LC50. More sophisticated assessments might use a fathead minnow to green sunfish extrapolation model or a green sunfish population model to relate the measurements to the assessment endpoint.

Measurement endpoints may be measured in the field or laboratory. Field measurements from monitoring or survey programs indicate what effects are occurring on a site. Laboratory measurements can be used to predict field effects or to provide evidence of causality for observed field effects. Measurement endpoints are typically simple statistical or arithmetic summaries of the measurement results. Examples are the LC50, a point on a regression line fitted to concentration-response data, and the relative abundance measures derived from field survey data.

In an unfortunately large number of monitoring programs, there are measurement endpoints, but the assessment endpoints are not clearly defined. In effect, the assessment endpoints are: "Are the things that we are measuring changing?" or "Are the things that we are measuring different on and off the site?" Without a better definition of why measurements are being taken, time and effort are wasted. If one monitors any aspect of the environment long enough, change will be seen; and if any two sites are sampled intensively enough, they will be found to differ. Minute changes or differences may be statistically significant but not environmentally significant. A clearly defined assessment endpoint not only indicates what is worth measuring, but also how intensively it must be measured.

The remainder of this document is concerned with the various sorts of measurements that can be performed for ecological assessments of hazardous waste sites. The purpose of this chapter is to make the assessor aware of the need to decide what is being assessed (i.e., to chose explicit assessment endpoints) before deciding what to measure.

This document does not describe methods for performing risk assessments. That is, it is not concerned with prediction of future effects or with optimization of the remedial actions. However, if the Superfund process proceeds beyond the activities described in this document, the effects of alternate remedial actions will have to be predicted and the remedial design selected in part on these predictions. If the measurements made for the ecological assessment are to be useful in this risk assessment and risk management process, then the assessment and measurement endpoints should be selected so as to be useful for prediction and relevant to the selection of remedial actions. Otherwise effort will have been wasted and the risk assessment will be impeded or impaired.

## 2.3 CRITERIA FOR ENDPOINTS

### 2.3.1 Assessment Endpoints

Criteria for a good assessment endpoint are listed in Table 2-1. First, an assessment endpoint should have social relevance; that is, it should be an environmental characteristic that is understood and valued by the public and by decision makers. In ecological assessments, the most appropriate endpoints often are effects on valued populations such as crops, trees, fish, birds, or mammals. This is not to say that species and other environmental attributes that are not publicly valued or understood have no place in ecological assessment. Rather, if species that are not socially valued are particularly susceptible, then their link to valued species or other valued environmental attributes must be explicitly demonstrated.

**Table 2-1. Characteristics of Good Assessment Endpoints**

| |
|---|
| Social relevance |
| Biological relevance |
| Unambiguous operational definition |
| Measurable or predictable |
| Susceptible to the hazard |
| Logically related to the decision |

It is desirable that the assessment endpoint have biological relevance. The biological significance of an effect is a function of its implications for the next higher level of biological organization. For example, the significance of infertility of individuals is determined by the resulting population reduction, and the significance of the loss of a major grazing species is determined by the ability of other grazers to functionally substitute for the lost species, thereby sustaining the community structure. Biomarkers are biologically significant if they indicate that individuals are being affected. However, some markers are also a part of adaptation to varying environmental conditions, which may have no long-term implications for whole organism performance. Biological significance may not correspond to societal significance. The abundance of peregrine falcons has clear societal significance and is a worthy assessment endpoint on that basis, but has no apparent biological significance.

Assessment endpoints should have unambiguous operational definitions so that they can be related to measurements. Phrases such as "ecosystem integrity" and "balanced indigenous populations" reflect concerns for a good natural environment.

Although they are suitable concepts for contemplation by the risk manager, they are not suitable subjects for assessments because they can not be measured or modeled from any measurement. Without well-defined endpoints, the ecological assessment will not provide useful insight for environmental decisions associated with the hazardous waste site. A complete operational definition of an assessment endpoint requires a subject (e.g., bald eagles or endangered species in general) and a characteristic of the subject (e.g., local extinction or a percentage reduction in range).

Assessment endpoints should be measurable or predictable from measurements. Assessment requires toxicity tests and statistical models for summarization and extrapolation of test results, measurements of responses of similar systems to similar hazards, or mathematical models of the response of the system to the hazard. An endpoint that cannot be tested, measured, or modeled cannot be assessed except by expert judgment. For example, responses of fish are good assessment endpoints because fish population and community characteristics are easily measured in the field, routine toxicity tests are available, and models are available to relate laboratory test species in the field.

The assessment endpoints chosen for a particular assessment must be susceptible to the hazard being assessed. Susceptibility results from a potential for exposure and responsiveness of the organisms or other entities to the exposure. In some cases, susceptibility will be known in advance because it prompted the assessment. In other cases, where a novel hazard is involved, or the causal linkage between the putative hazard and the observed damage is unclear, establishing susceptibility will be a goal of the assessment. This criterion is obviously situation-specific and will not be discussed further.

Finally, the assessment endpoints should bear some logical relationship to the environmental decisions of concern. For example, rates of soil processes may be considered as an assessment endpoint, but what does a decreased carbon mineralization rate mean when the potential remedial actions are capping the soil or incinerating it? In contrast, effects of leachate from the soil on aquatic communities are relevant.

Seriousness of effects has been mentioned in other discussions of endpoints (e.g., AMS 1987), but is excluded here as inappropriate. This criterion includes severity, reversibility, and extent. If an endpoint has societal and biological significance, then it should not be excluded simply because more serious effects are possible. Rather, both serious but low probability endpoints and less serious but potentially high probability endpoints should be assessed so that they can be considered and balanced in the risk management process.

### 2.3.2 Measurement Endpoints

Criteria for a good measurement endpoint are listed in Table 2-2. A measurement endpoint must correspond to or be predictive of an assessment endpoint. The environmental sciences literature is replete with examples of traits that were measured in the laboratory or field, but which could not be explicitly translated into a societally or biologically important environmental value. If the endpoint of a measurement does not correspond to an assessment endpoint, it should be correlated with an assessment endpoint, or should be one of a set of measurement endpoints that predict an assessment endpoint through a statistical or mathematical model. If this is not possible, then the measurement endpoint or suite of measurement endpoints should be protective; that is, they should be so sensitive and inclusive of the hazardous processes on the site that if they are not affected, nothing will be affected.

Table 2-2. Characteristics of Good Measurement Endpoints

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Corresponds to or is predictive of an assessment endpoint
Readily measured
Appropriate to the scale of the site
Appropriate to the exposure pathway
Appropriate temporal dynamics
Low natural variability
Diagnostic
Broadly applicable
Standard
Existing data series

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Measurement endpoints must be readily measurable. That is, it should be possible to quickly and cheaply obtain accurate measurements using existing techniques and personnel.

Measurement endpoints must be appropriate to the scale of the pollution, physical disturbance, or other hazard. It would be inappropriate to use the productivity of a deer population to assess the effects of a 1-hectare waste site, but it might be appropriate to use this index for a large complex of waste sites.

Measurement endpoints must be appropriate to the exposure pathway. The organisms or communities that are measured should be exposed to the polluted media and should have the same routes of exposure in approximately the same proportions as assessment endpoint organisms or communities. When such matching is not possible, then organisms that have the highest exposure should be used. For example, at sites where soil is contaminated, burrowing rodents have higher exposures than rodents that use surface runs and nests (McBee 1985).

Measurement endpoints should have appropriate temporal dynamics. If the hazard is episodic, then the measured response should be persistent so that evidence of effects will still be apparent after the event. For example, fish kills are apparent after pollution episodes, but behavioral responses tend to recover rapidly. Waste sites are generally thought of as sources of chronic exposure, but acute exposures may result due to spills (e.g., drum failures, overflowing sumps, or flushes of leachate following storms) and to movement of leachate to or near the surface (e.g., rainwater filling old sumps or waste trenches and creating "bathtubs" of leachate in the slumped surface). Also, stress markers (physiological indicators of stress) should not respond so rapidly that they increase due to the stress of capture.

Measurement endpoints should have low natural variability. Responses that are highly variable among individuals or across space and time are difficult to interpret when used to measure pollution effects. As a result, either the effects are masked or large numbers of replicates must be used. For example, fecundity is more sensitive to most pollutants than mortality in fish, but fecundity is highly variable among individual females, so fecundity effects are hard to distinguish in toxicity tests (Suter et al. 1987). The importance of variability depends on the relative scales of the variance and the measurements. For example, most pollution effects studies address effects on the scale of years, so diurnal variance is irrelevant, and variance due to climatic trends on the scale of hundreds to thousands of years is not detected.

It is desirable for measurement endpoints to be diagnostic of the pollutants of interest, to the extent that they have been identified. For example, concentrations of adrenal corticoids are indicators of stress in general; DNA single-strandedness is indicative of genotoxins; and DNA adducts of benzo[a]pyrene (BAP) are indicative of DNA damage by BAP (DiGiulio, this volume; McCarthy et al. in press).

It is desirable for measurement endpoints to be broadly applicable to allow comparison among sites and regions. For example, armadillos are probably good monitors of soil pollutants because they burrow and feed on soil and litter invertebrates. However, they occur in a small portion of the United States, whereas mice of the genus Peromyscus are ubiquitous.

Measurement endpoints should be standardized to assure precise, replicable results and to permit interpretation of results in terms of previously reported effects. Methods that have been standardized for toxicity testing or monitoring fulfill both of these needs. Methods that are standard in research or in some applied field other than toxicology (e.g., nitrification rates) fulfill the need for replicable results, but are difficult to interpret because there is no data base of toxic effects. Standard methods and endpoints for toxicity testing are readily available for a variety of aquatic organisms, for some terrestrial animals, for a few plant responses, and for a few microcosms and mesocosms. Sources include the American Society for Testing and Materials (ASTM), American Public Health Association (APHA), Organization for Economic Cooperation and Development (OECD), and U.S. Environmental Protection Agency (EPA). Standard methods for measuring pollutant concentrations in the environment are available from the same organizations. Methods for monitoring biota are much less standardized, and the few existing standards (e.g., APHA 1985, ASTM 1987) are not as widely used.

Finally, it would be desirable to use an endpoint that is already being measured so that there is a baseline from which to estimate background levels, variability, and trends. There is the additional advantage that data from an ongoing monitoring or testing program is free. This is seldom possible for waste sites, but there are areas, such as federal reservations, where biological monitoring precedes a CERCLA assessment.

## 2.4 POTENTIAL ASSESSMENT ENDPOINTS

Potential assessment endpoints for ecological risk assessments are listed in Table 2-3. They are arranged in terms of the standard ecological hierarchy, but the levels are not distinct. Endpoints are listed in the lowest hierarchical level to which they are appropriate. For example, massive mortality is listed under population, but can also occur within a community or region. The listed assessment endpoints are actually classes of endpoints; an endpoint for a real assessment would specify an entity and characteristic (e.g., kills of more than 100 fish of any species). Even at this level of generality, any list of endpoints will be incomplete. Anyone can imagine other assessment endpoints that may be useful in specific cases. The listed endpoints were chosen to have generic utility.

Table 2-3. Potential Assessment Endpoints

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- I. Population
  - Extinction
  - Abundance
  - Yield/production
  - Age/size class structure
  - Massive mortality
- II. Community
  - Market/sport value
  - Recreational quality
  - Change to less useful/desired type
- III. Ecosystem
  - Productive capability
- IV. Human health concerns
  - Contamination
  - Gross morbidity

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### 2.4.1 Population

Population-level assessment endpoints are generally the most useful in local assessments because (1) responses at lower levels (i.e., organismal and suborganismal) maybe perceived as having less social or biological significance (actions may be taken to protect individuals of endangered species but only because it is prudent in light of the precarious state of the population), (2) populations of many organisms have economic, recreational, aesthetic, and biological significance that is easily appreciated by the public, and (3) population responses are well-defined and more predictable with available data and methods than are community and ecosystem responses. The remainder of this discussion will refer to populations of socially or biologically important species.

The most drastic population-level effect is extinction; it is well-defined and potentially has great societal and biological significance. It can be predicted with good success if the hazard is habitat loss and with moderate success if the hazard is toxic effects. Extinction can be monitored with relative ease for conspicuous species, and, on the scale of a typical waste site, it can be readily monitored for almost any macroscopic species. Anthropogenic local extinctions are relatively common as a result of direct toxic effects, loss of habitat, loss of competitive ability with more resistant species, or other indirect causes.

Yield, abundance, and production are expressions of the ability of a population to fulfill a biological or resource role. If the yield (e.g., harvestable production) of a resource population such as timber trees or sport fishery declines, the societal significance is readily apparent. Abundance of nonresource species also has societal importance if the species is missed. The biological significance of both abundance and production may be large or small depending on the role of the species and its natural variability. These attributes are well-defined. Although techniques exist to predict these quantitative population responses, their reliability is not well established. Effects of habitat modification on wildlife can be predicted using the U.S. Fish and Wildlife Service's habitat evaluation procedure (Division of Ecological Services 1980) and effects of pollutants can be predicted by applying the effects observed in toxicity tests to population models (Barnthouse et al. 1987, and in press). These effects are easily measured for many species, but variance is often high.

Population-level endpoints are appropriate to waste site assessments when (1) individuals of a valued species occur on the site in communities receiving effluents from the site, or formerly occurred on the site in receiving communities, (2) those individuals are or were potentially exposed to waste chemicals, and (3) death or injury of those individuals are believed to cause significant effects on the population as a whole.

### 2.4.2 Community

Changes in the character of a biotic community can have major societal implications. If the market or sport value of a community changes, such as when a trout stream changes to a stream supporting only acidophilic bacteria due to acid leachate from mining waste, the societal implications are evident. Similarly, community changes such as severe eutrophication (possibly due to leaching of high phosphorous wastes) can diminish the recreational value of the community. There is a large body of literature on the economic value of recreation (Economic Analysis, Inc. 1987). Changes of community type that do not directly involve commercial, sport, or recreational values are also likely to be regarded as changing the utility or desirability of the community. However, the definition of what constitutes a significant negative change in a community type is often ambiguous. A moderate increase in the trophic status of a lake may increase production of desirable fish species, but diminish its value for swimming, boating, and aesthetic enjoyment, particularly for an oligotrophic lake.

Changes in community type are likely to have biological significance because large numbers of species and large areas are potentially involved. However, whether a change is biologically significant depends on the particular change and the community function under evaluation. For example, conversion of a mixed forest to a mowed grassland would decrease the movement of waste chemicals to the surface by plant roots but would decrease habitat for wildlife. It would also affect local hydrology by decreasing summer transpiration and increasing runoff.

Endpoints for most significant community transformations can be given good operational definitions. Examples include the conventional classification of lake trophic status and classifications of vegetation types.

Prediction of local community changes due to physical disturbances (e.g., converting a forest to lawn, or dredging a stream) is a trivial assessment problem. Effects on communities of additions of nontoxic pollutants (e.g., organic matter and nutrients from sludges) are reasonably predictable in aquatic systems, and there is a growing body of information on sludge and waste water disposal in terrestrial systems that can provide a basis for prediction. Effects of toxic chemicals on communities are not directly predictable. They can be inferred from information on toxicity to component taxa and knowledge of the relationship between taxa (O'Neill et al. 1982, West et al. 1980), but there is insufficient experience with this approach to evaluate its predictive power for community transformations. Microcosms and mesocosms are alternate means of assessing toxic effects in communities.

Community transformations that take the form of changes in vegetation are easily measured from ground surveys or aerial images. Changes in terrestrial animal communities and in aquatic communities require greater effort in sampling or observation, but present no conceptual problems.

Community-level endpoints are applicable to waste site assessments when a valued community exists on the site or receives effluent from the site and when the affected portion of the community is a significant portion of the entire community.

### 2.4.3 Ecosystem

The only ecosystem property that is generally useful for waste site assessment is productive potential. If productive use of the site is an option, then it is reasonable to consider the potential productivity of the site with and without remediation. This endpoint has social and biological significance and can be operationally defined if a future use is specified. It can be reasonably predicted either from the effects of the waste on production and estimates of the rate of loss of toxic chemicals from the system (assuming no restoration) or from the alternate restoration plans. Productivity is logically related to the decision. However, because remediation activities such as dredging streams, removing soil and vegetation, installing caps, and establishing a mowed grassland tend to reduce the productivity of a site, productivity considerations would often tend to be an argument against remediation.

### 2.4.4 Human Health Concerns

Contamination of populations by pollutants has societal significance if the organisms provide human food. This endpoint is well-defined by the FDA action levels. Contamination is readily predicted for aquatic organisms from concentrations in water and is relatively straightforward for terrestrial plants, but the complexity of exposure in terrestrial wildlife (food, water, air, and soil can all be important) makes prediction of body burdens very difficult.

The frequency of gross morbidity (tumors, lesions, and deformities) is societally significant because the public has come to interpret them as signs of pollution that may constitute a health threat, but they have little biological significance per se. Gross morbidity is not presently predictable, although deformities are observed in reproductive toxicity tests. Gross morbidity is readily measured because the conditions persist and can be evaluated by inspection of a sample of organisms.

## 2.5 MEASUREMENT ENDPOINTS

Potential measurement endpoints for waste site assessments are listed in Table 2-4. As with the assessment endpoints, these are general classes of endpoints. For example, actual measurement endpoints for individual mortality include median lethal concentration (LC50), the threshold for mortality in a cohort (LC01), the no observed effect level (NOEL) for mortality, and the number of dead individuals observed following a pollution episode. It is more difficult to generalize about the utility of measurement endpoints because the ability to measure an environmental characteristic and its relation to the spatial, temporal, and other characteristics of the hazard are situation-specific.

### 2.5.1 Individual

The endpoints of nearly all toxicity tests are statistical summarizations of the responses of individual organisms. For example, the LC50 is a statistical estimate of the concentration at which the median individual dies. Death, reproduction, and growth can be related to population-level assessment endpoints by using population models based on the survival and reproduction of individuals (Barnthouse et al. 1987, and in press) and to population and ecosystem endpoints by using ecosystem models (O'Neill et al. 1982, Bartell et al. 1987). Conventional laboratory tests are easily conducted, have reasonably low variability, are broadly applicable, are highly standardized, and can have appropriate temporal dynamics. Because exposure and other conditions are controlled, diagnostic effects are not needed. While the use of more than one test is advocated, it is important to select tests that relate to exposures on the site rather than using a battery of tests that are quick and convenient (e.g., Porcella 1983). For example, Daphnia tests of soil leachate when it is not polluting surface water or earthworm tests of desert soils provide no evidence concerning the magnitude or nature of ecological effects. Tests of plants and aquatic organisms typically have appropriate modes of exposure, but wildlife dosing or dietary tests are difficult to relate to wildlife exposure at most waste sites.

**Table 2-4. Potential Measurement Endpoints**

Individual
- Death
- Growth
- Fecundity
- Overt symptomology
- Biomarkers
- Tissue concentrations
- Behavior

Population
- Occurrence
- Abundance
- Age/size class structure
- Reproductive performance
- Yield/production
- Frequency of gross morbidity
- Frequency of mass mortality

Community
- Number of species
- Species evenness/dominance
- Species diversity
- Pollution indices
- Community quality indices
- Community type

Ecosystem
- Biomass
- Productivity
- Nutrient dynamics

Overt symptomology (visible effects such as spinal deformities in fish and chlorosis of plant leaves) and biomarkers (biochemical, physiological, and histological indicators of exposure or effects) are potentially diagnostic and measurable in field-collected organisms. Handbooks are available for attributing visible plant injury to specific air pollutants (Jacobson and Hill 1970; Malhotra and Blauel 1980). Overt symptomology and biomarkers, as well as behavioral responses, currently cannot be used to predict assessment endpoints even though they have clear implications for the health of organisms. There are currently no quantitative models that relate symptoms or biomarkers to higher-level effects. However, many biomarkers are diagnostic of exposure to particular classes of chemicals (e.g., metallothioneins for metal exposure) or for specific chemicals (e.g., DNA adducts of specific mutagenic chemicals) (DiGiulio, this volume; McCarthy et al. in press). In addition, tissue concentrations of accumulated chemicals are diagnostic of exposure to those chemicals, and, for most metals and some other chemicals, body burdens associated with effects are available in the literature. Both overt symptomology and tissue concentrations can be related to human health concerns. The variance of overt symptoms, biomarkers, and tissue concentrations depends on the chemical, marker, or symptom being measured. Only the methods for measuring tissue concentrations have been standardized.

Behavioral responses are difficult to measure in the laboratory and are even more difficult to measure in the field. They are not diagnostic or standardized, and, except for avoidance of the pollutant, tend to be difficult to interpret.

### 2.5.2 Population

The conventional population parameters (occurrence, abundance, age structure, birth and death rates, and yield) are poor subjects for laboratory tests, but are popular components of ecological field studies. They are directly interpretable in terms of assessment endpoints for valued populations. Occurrence and abundance are easily measured, but age structure is difficult to establish for many species. Birth rates, death rates, and yield are difficult to establish for many species (excluding annual plants) in short field studies. The scale of population responses is appropriate for very large waste sites or for populations with small ranges. Otherwise, movement of individuals and propagules onto or off of the site will obscure effects. In some cases, the waste site will constitute a habitat island with distinct populations, in which case the populations are automatically scaled to the site. Population responses have good temporal dynamics in that they integrate chronic and acute exposures. Their variability depends on the species. They are not diagnostic, however, and the requirement of a valued species on the site limits the applicability of population-level endpoints. Methods for population surveys are not standardized, but there are generally accepted methods applicable to most species.

The frequency of mass mortalities, and the frequency and nature of overt morbidity correspond to assessment endpoints. Overt morbidity is readily measured in the field for most vertebrates; however, mass mortalities are unlikely to occur during a field survey, so local residents or agencies must be the source of data. Frequencies of overt morbidity are quite variable and care must be taken in diagnosis of lesions and tumors to distinguish effects of toxicants from those of parasites and mechanical injury. These endpoints are not standardized and, with the possible exception of fish kills, are unlikely to be interpreted through the use of existing data.

### 2.5.3 Community

The most commonly used community characteristics in environmental monitoring are the number of species, species evenness, and species diversity. They are popular because they conveniently summarize the data generated by biotic surveys. They are easily measured, appropriate to the scale of the site, and they temporally integrate acute and chronic exposures. For most macroscopic flora and fauna, they have reasonably low variance, but the evenness and diversity of invertebrates tend to be high. They are broadly applicable, but not diagnostic or well standardized; some standard methods for community sampling exist (APHA 1985, ASTM 1987).

The problem comes in relating these numbers to assessment endpoints. If the nature and aspect of the community has not been affected, then changes in number, evenness, and diversity must be interpreted in terms of the species that have appeared, disappeared, or changed in relative abundance as a result of the presence of the waste. In other words, the assessment must shift to the population level because the number and diversity of species is no longer believed to confer stability or any other biological value (Goodman 1975). Certainly, the increase in species number and diversity that results from colonization of disturbed areas by weedy species is not valued or of great consequence. If the nature and aspect of the community has been changed by the presence of the waste, then number, evenness, and diversity numbers are simply adjuncts to the description of the changed community type. In many cases, intensive sampling and data summarization will not be necessary to describe community changes. A quick survey can establish that contaminated soils are entirely or nearly devoid of vegetation or that a stream draining a waste site is barren of macroorganisms. Although they are not sensitive, such descriptions of gross community changes are clearly good measurement endpoints where they are applicable.

Another type of community-level endpoint is the index of community quality, which may be indicative of pollution effects or of habitat quality in general. The best example of a community pollution index is the saprobic index (Hynes 1960). This index arrays aquatic communities with respect to conventional organic pollution (i.e., sewage and similar effluents) which predictably replace one set of species with another. Such indices are unlikely to be useful at waste sites, and it is unlikely that useful new pollution indices can be devised for waste sites because wastes are unlikely to have a suitably stereotypic effect. Indices of generic community quality, such as the index of biological integrity (IBI) (Karr et al. 1986), show promise as indicators of the state of communities because they are sensitive to physical habitat quality as well as to pollution. In addition, they have been applied to water quality assessments in contexts other than HWS evaluations. All of these community quality indices, like diversity indices, reduce to one number the information obtained from a biotic survey. Therefore, they do not indicate how two sites differ and provide no evidence as to the cause of the difference. However, if an index like the IBI is well characterized for a region, then it can be used to indicate how waste site effects compare to effects of other disturbances in similar communities. For most regions and community types, appropriate indices and baseline data are not currently available.

The indicator species concept is conceptually similar to community indices in that they are intended to describe the state of communities relative to anthropogenic effects. The presence or abundance of a species that is thought to be either pollution-sensitive or pollution-tolerant is used to indicate the status of a community. Like the saprobic index, indicator species have been effective for assessing oxygen-demanding pollution, but not for other types. Therefore, an indicator species may not reliably define effects of hazardous waste sites, but within site-specific contexts may contribute to the ecological assessment.

### 2.5.4 Ecosystem

Ecosystem properties relate to the exchange of energy and nutrients among functionally defined groups of organisms and between organisms and the environment. The most commonly measured ecosystem properties are biomass of the system or its components (e.g., trophic levels), productivity of the system or its components (e.g., primary and secondary production), and nutrient dynamics (e.g., nitrogen mineralization rates). These do not correspond to any assessment endpoint, but all relate to the productive capability of a site. In particular, the realized productivity of a site is an estimator of its productive capability, which may or may not be relevant to its post-restoration potential. Productivity is more relevant to affected off-site ecosystems, but, in any case, ecosystem or trophic level production is less socially meaningful than production of valued populations. Soil processes would seem particularly promising because the waste chemicals typically occur at the greatest concentration in soil. However, the complexity of soil processes, including competition between natural processes and degradation of the waste, and the wide range of organisms involved make interpretation difficult (Suter and Sharples 1984). Ecosystem properties can be difficult to measure on site, tend to be highly variable, are not diagnostic, and are difficult to interpret, but are broadly applicable. No standard methods exist for measuring toxic effects on ecosystem processes in the field, but the EPA has recently adopted laboratory microcosm protocols that include some measurements of ecosystem processes (Office of Pesticides and Toxic Substances 1987).

## 2.6 ASSESSMENT GOALS AND ASSESSMENT ENDPOINTS

Although the primary focus of this document is on selecting measurement endpoints and performing measurements, it is critical to keep assessment endpoints and their relation to the decision making process in mind. The point of the ecological assessment is not to find out if anything ecological has been, is being, or could be affected. Rather, it is to determine whether ecological effects have any relevance to the choice of remedial action or other decisions. Is any socially valued ecological entity being significantly affected in a way that can potentially be remediated? In some cases the answer is clearly no. It would not be appropriate to go through an ecological assessment process at most urban sites where there are no significant ecological values, at residential sites where ecological values are minor relative to potential human effects, or at sites where only deep geologic strata and ground water are contaminated. On the other hand, an ecological assessment may reveal that in spite of the waste, a valuable and viable community exists on the site that would be destroyed by conventional remedial actions. Therefore, in choosing endpoints the assessor should consider the nature of the site, its current and potential ecological state, the nature and dynamics of the wastes, and the potential remedial actions.

The problem of scale of effects is particularly acute in assessments of waste sites, because sites tend to be small. Scale is not such a problem for human health assessment because individual humans are valued so a site that includes a single human resident is important. If endangered species are not an issue, plants and animals are generally not valued biologically as individuals so it is necessary to consider the magnitude of effects on a waste site relative to entire populations, communities, or regions. An entire distinct microbial community can exist under a single waste drum, and a distinct rodent population can exist on a waste site such as Love Canal, but these communities may not have social significance. On the other hand, socially significant populations, such as birds and medium to large mammals, typically have populations that occupy large areas and may not be significantly affected by toxic effects on a few individuals on a waste site. Similarly, most plant community types occupy large areas relative to the scale of a typical waste site.

Therefore, ecological assessment effort should be concentrated on situations where considerations of scale does not limit the significance of effects. One such situation is large complexes of waste sites such as an oil field with numerous sumps, spills of toxic materials, oil spills, land farms, and landfills spread over several square kilometers. Another is where a waste site is able to significantly influence all or a major portion of an off-site community. For example, plans for oil shale development in the Piceance Basin, CO., involved filling the upper ends of canyons with retorted shale, which would have resulted in associated trout streams being fed by waste leachate and runoff (Suter et al. 1986). A third situation where scale is not a problem is use of a site by an endangered species such as the bald eagles at the Rocky Mountain

Arsenal. Injury of even a few individuals of an endangered species is not allowed because each individual is assumed to be important to the survival of the species.

In the case of large complexes of sites, two types of assessment endpoints might be appropriate. One type is the proportion of the community that has experienced severe effects, such as devegetation of the individual sites by persistent phytotoxic chemicals. This type of endpoint is readily measured and expressed at the community level. The other type is reductions in a population experiencing combined effects of habitat loss and toxic chemicals. This can occur either as members of the population move across the site, spending various amounts of time at variously contaminated locations and being exposed by various routes, or by integrating the effects of a mosaic of individuals inhabiting clean or contaminated habitat. These population effects are more difficult to assess because changes in the population as a whole are difficult to attribute to the waste sites, and effects on individuals inhabiting the waste sites must first be identified and then extrapolated to the population level.

The situation of a waste site dominating an off-site community is more straightforward. The choice of assessment endpoint depends on the valued attributes of the affected system. In the oil shale example, the assessment endpoint would be trout production and the measurement endpoints might be trout density, indices of trout production (e.g., age to weight relationships), and trout prey base.

In the case of an endangered species, the assessment endpoint would be reduction in the recovery rate of the species from its endangered status. Population parameters of an endangered species are likely to be poor measurement endpoints because the number of individuals is likely to be low and the species is likely to be far from equilibrium with its environment. Measurement of effects is complicated by the inability to destructively sample the subjects. Sampling for body burdens or biomarkers is largely limited to food species or to surrogate species that have similar ecologies, physiologies, and exposure patterns to the endangered species. In general, community and ecosystem properties are of interest not so much for their ability to support the endangered species as for their role in causing exposure of the endangered species to waste chemicals.

## 2.7 REFERENCES

American Management Systems, Inc. (AMS). 1987. Review of the literature on ecological end points. Report to the Office of Policy, Planning and Evaluation, U.S. Environmental Protection Agency, Washington, DC.

American Public Health Association (APHA). 1985. Standard Methods for the Examination of Water and Wastewater. APHA, Washington, DC.

American Society for Testing and Materials (ASTM). 1987. Annual Book of ASTM Standards: Water and Environmental Technology. American Society for Testing and Materials, Philadelphia, PA.

Balcomb, R. 1986. Songbird carcasses disappear rapidly from agricultural fields. Auk. 103:817-820.

Barnthouse, L.W., G.W. Suter II, A.E. Rosen, and J.J. Beauchamp. 1987. Estimating responses of fish populations to toxic contaminants. Environ. Toxicol. Chem. 6:811-824.

Barnthouse, L.W., G.W. Suter II, and A.E. Rosen. In press. Inferring population-level significance from individual-level effects: An extrapolation from fisheries science to ecotoxicology. In: G.W. Suter II and M.E. Lewis, eds., Aquatic Toxicology and Hazard Assessment, Eleventh Volume, American Society for Testing and Materials, Philadelphia, PA.

Bartell, S.M., R.H. Gardner, and R.V. O'Neill. 1987. An integrated fate and effects model for estimation of risk in aquatic systems, pp. 261-274. In: Aquatic Toxicology and Hazard Assessment, Vol. 10. American Society for Testing and Materials, Philadelphia, PA.

Division of Ecological Services. 1980. Habitat evaluation procedure (HEP). ESM 102. U.S. Fish and Wildlife Service, Washington, DC.

Eberhardt, L.L. 1976. Quantitative ecology and impact assessment. J. Environ. Manage. 4:27-70.

Economic Analysis, Inc. 1987. Measuring damages to coastal and marine national resources: Concepts and data relevant for CERCLA Type A Damage Assessments, PB87-142485. National Technical Information Service, Springfield, VA.

Goodman, D. 1975. The theory of diversity-stability relationships in ecology. Quarterly Review of Biology. 50:226-237.

Hynes, H.B.N. 1960. The Biology of Polluted Waters. Liverpool University Press, Liverpool, UK.

Jacobson, J.S., and A.C. Hill, eds. 1970. Recognition of Air Pollution Injury to Vegetation: A Pictorial Atlas. Air Pollution Control Association, Pittsburgh, PA. 102 pp.

Karr, J.R., K.D. Fausch, P.L. Angermeier, P.R. Yant, and I.J. Schlosser. 1986. Assessing biological integrity in running waters: A method and its rationale. Illinois Natural History Survey Special Publication No. 5, Illinois Natural History Survey, Champaign, IL. 28 pp.

Malhotra, S.S., and R.A. Blauel. 1980. Diagnosis of air pollutant and natural stress symptoms on forest vegetation in western Canada. Northern Forest Research Center, Edmonton, Canada. 84 pp.

McBee, K. 1985. Chromosomal aberations in resident small mammals at a petrochemical waste dump site: A natural model for analysis of environmental mutagens. Ph.D. dissertation, Texas A&M University, College Station, TX.

McCarthy, J.F., L.R. Shugart, and B.D. Jimenez. In press. Biological markers in wild animal sentinals. In: Bioindicators of Exposure and Effect, Eighth ORNL Life Sciences Symposium.

Office of Pesticides and Toxic Substances. 1987. Toxic Substances Control Act Test Guidelines, OPTS-42095. 40 CFR, Parts 796-797.

O'Neill, R.V., R.H. Gardner, L.W. Barnthouse, G.W. Suter II, S.G. Hildebrand, and C.W. Gehrs. 1982. Ecosystem risk analysis: A new methodology. Environ. Toxicol. Chem. 1:167-177.

Porcella, D.B. 1983. Protocol for bioassessment of hazardous waste sites, EPA/600/2-83-054. U.S. Environmental Protection Agency, Corvallis, OR.

Suter, G.W., II, and F.E. Sharples. 1984. Examination of a proposed test for effects of toxicants on soil microbial processes, pp. 327-344. In: D. Liu and B.J. Dutka eds. Toxicity Screening Procedures Using Bacteria. Marcel Dekker, Inc., New York, NY.

Suter, G.W. II, et al. 1986. Environmental risk analysis for oil from shale, ORNL/TM-9808, Oak Ridge National Laboratory, Oak Ridge, TN.

Suter, G.W., II, A.E. Rosen, E. Linder, and D.E. Parkhurst. 1987. Endpoints for responses of fish to chronic toxic exposures. Environ. Toxicol. Chem. 6:793-809.

U.S. Department of Interior. 1987. Type B Technical Information Document. Injury to Fish and Wildlife Species. CERCLA Project 301. Washington, DC.

West, D.C., S.B. McLaughlin, and H.H. Shugart. 1980. Simulated forest response to chronic air pollution stress. J. Environ. Qual. 9:43-49.

# CHAPTER 3

# ASSESSMENT STRATEGIES AND APPROACHES

By

Joan P. Baker, Kilkelly Environmental Associates, Raleigh, NC.

## 3.1 INTRODUCTION

Careful selection of the specific techniques and measures to be applied at a hazardous waste site (HWS) will maximize the value of an ecological assessment. The optimal design and methods for an ecological assessment vary depending upon the characteristics of the HWS and the specific objectives and issues of concern. Given the diversity of environmental conditions and problems at HWSs, a single best strategy or design for ecological assessments, appropriate for all sites, cannot be defined. Instead, to aid in selecting the best approach for a given HWS, this chapter provides a general discussion of the alternative methods or "tools" available, and the types of information contributed by each.

## 3.2 REVIEW OF EXISTING INFORMATION FOR THE SITE

The more that is known about conditions at the HWS, the more efficiently one can conduct an ecological assessment. The first step in the design of the ecological assessment, therefore, should be a compilation and review of this existing information for the site. Examples of relevant information include the following:

- **Site history** -- Information on prior industrial activities at the site (e.g., operational history for the Rocky Mountain Arsenal) provides insight into the nature, sources, and extent of site contamination.
- **Chemistry data** -- As part of the HWS evaluation process, contaminant concentrations in local soils, sediments, and waters will be determined. As noted in Chapter 1, ecological assessments involve the integration of these chemical data with results from the biological assessment methods described in this document. This integration will only occur if the chemical sampling and biological sampling are closely coordinated. For example, collection of

chemical and biological samples must be done at common sites for direct data comparisons. If chemical sampling has occurred at the HWS prior to initiation of the ecological assessment, results from these studies will play a major role in the development of the sampling design for the ecological assessment by identifying "hot spots" or gradients of contamination that represent important locations for biological sampling and testing. Results from biological sampling also may aid in optimizing the design for further chemical sampling program.

- **Results from fate and transport models** -- Models of contaminant movement and transformation provide insight into the extent and distribution of potentially toxic substances at the HWS, both on site and off site. Model results may identify locations and ecosystem components (e.g., soils and associated soil organisms, or surface waters and aquatic biota) most likely to be impacted. Results from the ecological assessment may, in turn, be useful in the development and testing of fate and transport models. Thus, again, coordination of these activities should be given a high priority.

- **Existing ecological data** -- Historical data for the HWS, or recent ecological studies of similar, nearby ecosystems not affected by the HWS, may be used to define natural, background conditions expected at the HWS. If such reference data do not already exist, they must be collected as part of the ecological assessment process. In addition, the design of the ecological assessment should take full advantage of any prior studies of ecological effects at the HWS.

Since the data collected as part of an ecological assessment can benefit the design and interpretation of other components of the HWS evaluation, ecological studies should be initiated as early as possible in the HWS evaluation process. Procedures for incorporating other sources of information within the ecological assessment design and analysis are discussed further in Chapters 4 (Field Sampling Design) and 9 (Data Interpretation).

## 3.3 INITIAL SITE VISIT

The second step in an HWS ecological assessment involves a visit to the site by a trained ecologist familiar with ecological community types in the region and with experience in HWS evaluations. The primary objectives of this initial site visit are to (1) identify the basic environmental (physical, chemical, and biological) characteristics of the site and (2) develop a qualitative map of the major types and status of ecological communities at the HWS. Little, if any, quantitative sampling is required (or recommended) at this stage; both the map and site characterization are based largely on a visual assessment of site conditions. Off-site habitats should also be examined if off-site effects are suspected to occur. The following environmental features should be noted and, if appropriate, mapped:

- **Major landscape features** -- site topography and the distribution of major habitat types, e.g., grasslands, forests, lakes, streams, wetlands.
- **General physical and chemical characteristics of the terrestrial environment** -- soil type(s) and local geology.
- **General physical and chemical characteristics of the aquatic environment** -- lake area and depth, stream size and flow, types of bottom substrate, temperature, water clarity, and general water quality parameters such as conductivity, salinity, hardness, pH, temperature, alkalinity, and dissolved oxygen levels.
- **Vegetation types** -- identification of dominant species and classification of the major vegetation community types.
- **Occurrence of important terrestrial and aquatic animals** -- qualitative observations of birds, mammals, fish, stream benthos, and other animals inhabiting the HWS, or the apparent absence of organisms considered typical of the HWS habitat type(s).
- **Occurrence of areas of contamination and ecological effects** -- locations of obvious zones of chemical contamination and ecological effects, ranked by apparent severity (e.g., ranked on a scale of 1 to 3, where 1 = obvious effects, 2 = possible effects, and 3 = no observed effects).

As part of these initial site characterization activities, it may also be appropriate to collect selected soil, sediment, and water samples for assessment of acute toxicity (see Chapter 6). Sites for sample collection should be selected subjectively in areas of obvious ecological effects or at locations where ecological effects are most likely to occur (based on prior chemical surveys or modeling). To the extent possible, samples should be collected from each major habitat type (i.e., terrestrial and aquatic habitats, soils, aquatic sediments, and surface waters).

## 3.4 DEVELOPMENT OF THE ASSESSMENT STRATEGY AND DESIGN

The existing site data and results from the initial site visit provide the basis for developing a site-specific assessment strategy and design. Important components of this plan include the following:

- **Specific objectives** -- The objectives of the ecological assessment should be clearly defined and should reflect both primary ecological concerns and the anticipated role of the ecological assessment in the HWS evaluation process and subsequent decision making.
- **Conceptual framework** -- Formulating the optimal design for an ecological assessment may be facilitated by developing a conceptual model for the site, including information on the movement and distribution of contaminants, likely interactions among ecosystem components, and expected ecological effects at the HWS, on site and off site.
- **Assessment and measurement endpoints** -- The assessment endpoints and corresponding measurement endpoints to be provided by the ecological assessment should be selected based on the criteria outlined in Chapter 2. The selected endpoints should match the specific objectives defined above.
- **Assessment methods** -- For each measurement endpoint, one or more of the methods outlined in Chapters 6 through 8 should be chosen as the optimal means for quantifying the response variable of interest.
- **Quality assurance/quality control** -- For each measurement endpoint, a data quality objective (DQO) must be defined, i.e., the measurement precision and accuracy required in order to satisfy the objectives of the HWS evaluation. In addition, procedures for monitoring and controlling data quality must be specified and incorporated within all aspects of the ecological assessment, i.e., during sample collection, processing, and analysis; data management; and data analysis. Data quality objectives and procedures for quality assurance/quality control are discussed further in Chapter 5.
- **Field sampling design** -- Statistical issues relating to design of the field sampling program (e.g., optimal sample size, procedures for sample selection) are discussed in Chapter 4.
- **Schedule** -- Typically, the entire HWS evaluation (including planning, data analysis, and report preparation) must be completed within 12 to 18 months. Thus, the ecological assessment may be subject to quite severe time constraints. On the other hand, some of the ecological methods, particularly field surveys, may be easier and more effective to do if conducted at certain times of the year. The schedule and time requirements for each aspect of the ecological assessment must be given careful consideration.

- **Data analysis plan** -- Prior to the collection of data, a specific plan for data analysis should be developed. By considering, immediately, the types of analyses and outputs anticipated, important components, confounding factors, and data requirements are less likely to be overlooked.

A tiered approach to an ecological assessment may be particularly effective. At each step, or tier, the decision is made whether to proceed and how best to proceed, based on the data collected up to that point. The tiers may be designed to reflect increasing levels of effort and/or different aspects of the overall HWS ecological evaluation. In the first instance, Tier 1 may consist of relatively crude, but rapid and inexpensive methods for evaluating the extent and severity of ecological effects. If severe and extensive effects are documented at this stage, there may be no need for additional data to quantify the problem at the HWS. On the other hand, if few or no effects are detected, it cannot be assumed that significant adverse effects are not occurring. Thus, it may be necessary to apply more sensitive and comprehensive methodologies, which are likely also to be more costly and time consuming, in a second tier of analyses.

Tiers may also be designed to address a series of questions regarding ecological conditions and effects at the HWS. In this case, results from the first tier feed directly into design of the second tier, and Tiers 1 and 2 into Tier 3, etc. For example, Tier 1 could involve field surveys to determine whether significant population-level effects on important organisms can be documented at the HWS (e.g., a significant reduction in the abundance of important game fish in receiving streams). If such effects are measured, of primary interest in Tier 2 would likely be the relationship, if any, between the observed field effects and the toxicity of contaminants at the HWS. One approach for Tier 2, therefore, would be to conduct aquatic toxicity tests using water samples collected along the gradient of effects observed in Tier 1. If no toxic response is measured, the population-level effect observed in the field survey may result principally from habitat degradation, rather than the presence of hazardous wastes at the site. In certain instances (e.g., if the initial site visit suggested no overt effects), it may be better to reverse the order of these tasks, asking first whether acute or chronic toxic effects can be demonstrated before conducting field surveys to quantify ecological status. Decisions regarding the optimal order for addressing assessment issues are likely to be site specific, depending on the nature of the site and existing information on the HWS.

The step-by-step, tiered approach is intended to maximize the efficiency of data collection, using the information obtained at each stage to optimize the design of the next stage. Typically, such an approach would require multiple trips to the HWS. The logistics of on-site sampling at an HWS, however, can be quite cumbersome. In such cases, the benefits derived from a tiered approach may be more than offset by the added costs and difficulties associated with additional site visits. A tiered approach may also require more time to implement, and thus may or may not be feasible within the time constraints of the HWS evaluation. Again, the optimal strategy for an ecological assessment would be site specific, depending on the complexity of the site, the difficulties and costs associated with obtaining access to the site, and the available time for data collection.

## 3.5 ASSESSMENT METHODS

The methods recommended for use in ecological assessments at HWSs are grouped into three major categories: (1) toxicity tests (see Chapter 6), (2) biomarkers (see Chapter 7), and (3) field surveys (see Chapter 8). Each of these basic methodologies contributes a different type of information to the HWS evaluation. As a result, all three must often be applied to gain a complete understanding of the ecological effects at an HWS. The following subsections provide an overview of the primary advantages, and also limitations, of each of these major categories of assessment methods. Similar discussions for specific recommended methods and procedures are presented in Chapters 6 through 8.

### 3.5.1 Toxicity Tests

Toxicity tests measure the effects of contaminated media from the HWS on the survival, growth, and/or reproduction of aquatic and terrestrial biota. Most often, samples of soil, sediment, or water are collected from the HWS and returned to the laboratory for testing with several standard laboratory test species. Toxicity tests can also be run in mobile laboratories or in situ, and with resident species from the site (see section 6.1).

The advantages and limitations of using toxicity tests in ecological assessments are reviewed in Table 3-1. Chemical analyses provide a measure of the total concentration of specific chemical compounds. Toxicity tests, on the other hand, provide an integrated index of the bioavailable toxic contaminants on the site. Furthermore, some toxic chemicals on a site may not be measured accurately in chemical analyses because of the complexity of the matrix or analytical detection limits. Thus, toxicity tests play an important role in and of themselves in site assessments, and potentially link the occurrence of contamination, as evidenced by an elevated chemical concentration, to biological effects. Toxicity tests are only an index, however, of the potential for population- or community-level effects at the HWS. Demonstration and quantification of ecological effects require field surveys.

### Table 3-1. Advantages and Limitations of Toxicity Tests in Ecological Assessments

| Advantages | Limitations |
|---|---|
| Measure of toxic conditions that can be linked to the presence of contaminants and hazardous wastes; an important assessment component needed to establish causality. | Measure of potential toxic effects on resident biota at the HWS; however, cannot always be directly translated into an expected magnitude of effects on populations in the field. |
| Results are an integrated index of bioavailable contamination, whereas chemical analyses measure only total concentrations of specific compounds. | Results are somewhat dependent on specific techniques, e.g., test species, water or soil quality, test duration, etc. |
| Results are specific to the location at which the sample was collected, thus they can be used to develop maps of the extent and distribution of bioavailable contamination and toxic conditions. | Ecological survey data also provide an integrated measure of effects for the entire HWS, and may be more useful for addressing certain assessment objectives. |
| Results are easily interpreted and amenable to QA/QC; within- and among-laboratory precision, estimates are already available for several tests. | Exposure conditions in toxicity tests are not directly comparable to field exposures; additional confounding variables and other stresses are important in the field. |
| Acute toxicity tests are relatively quick, easy, and inexpensive to conduct; results from acute tests are used as a guide in the design of chronic toxicity tests. | Acute tests are less sensitive measures of toxic conditions (relative to chronic tests or biomarkers); thus, the absence of an acute toxic response cannot be interpreted as the absence of a toxicity problem |
| Chronic toxicity tests are generally more sensitive than are acute tests, and can be used to define "no effect" levels; in addition, chronic tests provide a better index of field population responses and more closely mimic actual exposures in the field. | Chronic tests require more time and and expertise to conduct, yet still may not detect all sublethal effects. |

Results from toxicity tests are specific to the site of sample collection, and thus can be mapped to define gradients and zones of toxic conditions. Such maps, in addition to response surfaces of toxicity, can serve as a guide to the design of field surveys and other sampling programs. A close correspondence between spatial patterns of toxicity and spatial patterns of effects measured in field surveys provides strong evidence for the importance of toxic contaminants in controlling the status of ecological communities at the site.

Like chemical analyses, procedures for quality assurance and quality control for toxicity tests are fairly well established. Given standardized test conditions, as described in Chapter 6, results from toxicity tests are typically highly repeatable both within and among test laboratories.

Toxicity tests are generally classified as either acute (short-term) or chronic (long-term) depending on the length of exposure of the organism to the contaminated media. Acute toxicity tests are probably the best means for conducting a first-order assessment of the distribution and extent of toxic conditions at a site. They are relatively quick, easy, and inexpensive to conduct. On the other hand, acute tests tend to be less sensitive measures of toxicity than are chronic tests or biomarkers. Thus, the absence of an acute toxic response cannot be interpreted as the absence of a toxic problem. Chronic toxicity tests, while requiring additional time and expertise, may be needed to detect less severe, but still important, toxic effects. In particular, chronic toxicity tests may be used to define "no effect" levels, useful for evaluating the effectiveness of remediation programs.

Microbial systems, and methods relying on measurements of microbial activity, were treated somewhat separately in development of the recommended methodologies for ecological assessments. Although included within the chapter on toxicity testing (Section 6.4), some of these procedures could also be applied in field surveys; many assay the effects of contaminants on sensitive physiological and biochemical processes and thus could also be considered biomarkers.

The advantages and limitations of using microbial tests in ecological assessments are reviewed in Table 3-2. The advantages result principally from their small size and generally rapid response. Most of the tests described are quick, inexpensive, and easy to conduct, and require quite small sample volumes, an added advantage if the samples are to be transported from the field back to the laboratory. In addition, many of the microbial functional responses assayed represent important ecosystem processes and microbial tests have been applied in the field to evaluate these processes. Unfortunately, relatively little data are available on the effectiveness of these tests for measuring toxicity at HWSs.

**Table 3-2. Advantages and Limitations of Microbial Studies in Ecological Assessments**

| Advantages | Limitations |
|---|---|
| Tests are quick, inexpensive, and relatively easy to conduct, and require small amounts of sample. | Relatively little data are available on the responses of microbes to HWS contaminants. |
| Many of the response variables represent basic ecosystem processes. | Relationship between responses in small-scale tests and ecosystem processes has not been evaluated in the field. |

### 3.5.2 Biomarkers

The term "biomarkers" refers to the measurement of selected endpoints in individual organisms, typically physiological or biochemical responses, that serve as sensitive indicators of exposure to contaminants and/or sublethal stress. As used in this document, measures of bioaccumulation, i.e., chemical concentrations of contaminants in organisms, are considered a biomarker of exposure. Other examples of biomarkers of exposure and sublethal stress include the following: (1) concentrations of enzymes such as cholinesterases and delta-aminolevulinic acid dehydrase (delta-ALAD); (2) genetic abnormalities, e.g., DNA unwinding; (3) physiological responses, such as rates of gas exchange in plants; and (4) histopathological (e.g., occurrence of tumors) or skeletal abnormalities (see Chapter 7).

The advantages and limitations of using biomarkers in ecological assessments are reviewed in Table 3-3. An important advantage is their broad applicability. The techniques can be applied at many taxonomic levels (plants and animals) and the results have inferences that go beyond the organism(s) tested. Evidence for genotoxicity or disruption of basic physiological and biochemical processes based on biomarker analyses have relevance to assessments of potential hazards to human health.

Biomarkers can be measured in organisms collected from the field, reflecting "real-world" exposures, and in organisms exposed to contaminated media under more controlled conditions in the laboratory or in situ. Thus, biomarkers provide an important tool for comparing biological responses in the laboratory and in the field since the same methods can be applied in both environments. In addition, some tests are diagnostic of specific contaminants, and most tests provide some information on the mechanism of toxic response. All of these attributes aid in establishing causality for ecological effects in the HWS evaluation.

Table 3-3. Advantages and Limitations of Biomarkers in Ecological Assessments

| Advantages | Limitations |
|---|---|
| Broadly applicable; a measure of biological response that crosses taxonomic lines, including inferences to potential human health effects. | Relationship between biomarkers and population-level effects in the field are not well defined. |
| Provides insight into the potential mechanisms of contaminant effects; in many cases, biomarkers are diagnostic of specific contaminants. | Biomarkers are still lacking for most of the compounds of interest at HWSs. |
| Can be applied in both the laboratory and field, providing an important linkage between laboratory toxicity tests and effects in the field. | Require particular care in sample handling as well as added time and expense. |
| For field samples, biomarkers provide an important index of bioavailability with "real-world" exposures. | For mobile species, difficult to define "exposure;" may require destructive sampling. |
| When applied correctly (i.e., a biomarker appropriate for the contaminants at the site) may be a very sensitive index of bioavailability and biological response. | Important to carefully define reference conditions, a problem common to all field studies. |

The major limitation in applying biomarkers in ecological assessments is the current lack of accepted, standardized, and tested markers for many of the HWS contaminants of interest. While a number of biomarkers are sufficiently developed for use at this time, many others are still under development and require further research. In addition, for most biomarkers, the relationship between a measured biomarker response and population-level effects has not been defined. Biomarkers are highly sensitive indices of exposure and sublethal response, but, within the context of an ecological assessment, their relevance is most evident when biomarker studies are conducted jointly with toxicity testing and field surveys.

### 3.5.3 Field Surveys

Field surveys involve the measurement of the structural and functional characteristics of populations and communities at the HWS. Recommended methods for field surveys are outlined in Chapter 8 for aquatic ecosystems (section 8.2), terrestrial vegetation (section 8.3), terrestrial vertebrates (section 8.4), and terrestrial invertebrates (section 8.5).

The advantages and limitations of using field surveys in ecological assessments are reviewed in Table 3-4. While toxicity tests may infer potential population- and community-level effects, field surveys are the only means for demonstrating actual population- and community-level effects at the HWS. Survey data identify the "problem" and the extent of the problem. Organisms are exposed in the "real world," and measured effects represent an integrated response to the temporal and spatial variations in exposure and contaminant concentrations in the field. With survey data alone, however, the causes for observed effects are difficult to determine. As noted in the preceding sections, causality is established best by a combination of approaches, including chemical sampling, toxicity testing, biomarkers, and field surveys.

Results from field surveys and measures of ecological status are often highly variable, reflecting the high degree of variability (both spatial and temporal) in natural communities and, in some cases (e.g., fish communities in lakes), the problems inherent in sampling the biological community. As a result of this high background variability, fairly extensive sampling may be needed to measure the ecological characteristics of interest with a sufficient level of precision to detect "effects" related to the HWS. Careful attention to sampling design (Chapter 4) is required to ensure that the survey results satisfy the objectives (and data quality objectives) of the HWS evaluation. Procedures for quality assurance/quality control exist for field surveys, but they are not nearly as well established or clear-cut as are protocols for other components of the ecological assessment.

Table 3.4 Advantages and Limitations of Field Surveys in Ecological Assessments

| Advantages | Limitations |
|---|---|
| Characterizes the basic ecology of the site, identifying important resident species and community types; based on results from the field survey, relevant species for use in toxicity testing and biomarker analyses can be identified. | Results from field surveys may be highly variable, requiring extensive sampling to measure ecological status with sufficient precision for detection of effects; as a result, the absence of a measurable effect cannot always be interpreted as no effect. |
| Potentially demonstrates definitive ecological effects in the field, delineating zones of effect and no apparent effect. | With survey data alone, causes for observed effects are difficult to determine. |
| Field responses integrate temporal and spatial variations in exposure and contaminant concentrations. | Results represent only a snapshot of the ecological status at the time of the survey. |
| Information on the status of terrestrial vegetation can be obtained from aerial photographs, eliminating the need to visit the HWS to survey terrestrial vegetation. | Procedures for QA/QC are not well established; difficult to measure precision and accuracy. |

## 3.6 SUMMARY

Key questions of interest for ecological assessments at HWS and recommended approaches for addressing these questions are summarized in Table 3-5.

Table 3-5. Recommended Approaches for Addressing Key Questions for Ecological Assessments at Hazardous Waste Sites

| Key Questions | Recommended Approach | Example Measurement Endpoints and Outputs |
|---|---|---|
| Have biological communities or populations, on site or off site, been measurably impacted at the HWS? | Field surveys | Occurrence and abundance of important species at the HWS relative to values for comparable reference areas. |
| Are soils, water, or sediments at the HWS contaminated? | Chemical analysis | Chemical concentrations of contaminants of concern, at the HWS, relative to values for comparable reference areas. |
|  | Toxicity tests | Toxic response to samples. |
| Are the contaminated soils, water, and sediments at the HWS toxic or hazardous to living organisms? | Acute and chronic toxicity tests<br>Biomarkers of sublethal stress | Percent survival or occurrence of biomarkers for organisms exposed to contaminated media for the HWS, relative to appropriate reference values. |
| Are organisms at the HWS exposed to these hazardous contaminants? | Biomarkers of exposure | Chemical concentrations of contaminants or frequency of occurrence of other biomarkers for organisms collected from the field at the HWS, relative to values for organisms from comparable reference areas. |
| Are the effects of biological communities and the populations at the HWS caused by the presence of hazardous wastes? | Use all of the above | Comparison of the spatial patterns for effects at the HWS measured with (1) field surveys of ecological status, toxicity testing with contaminated media, (2) surveys of biomarkers of exposure and sublethal stress, (3) chemical surveys, and (4) outputs from fate and transport modeling. |

# CHAPTER 4

# FIELD SAMPLING DESIGN

By

Donald L. Stevens, Jr., Eastern Oregon State College, La Grande, OR.

## 4.1 GENERAL STATISTICAL CONSIDERATIONS

Each hazardous waste site (HWS) considered for ecological assessment will, to some extent, present unique problems in sampling design and data analysis because of differences in site characteristics and potential contaminants. No single field sampling design can be suitable for every HWS. A competent statistician should always be consulted prior to designing any laboratory or field study and collecting data.

Field sampling activities must be coordinated between sample collection for chemical analysis, laboratory toxicity testing, and field survey activities. Sample collection and field survey activities should be coordinated in space and time. The following three types of information are necessary to establish a relationship between toxic wastes and ecological effects: (1) chemical analysis of the appropriate media are necessary to establish the presence, concentration, and variability of toxic chemicals; (2) ecological surveys are necessary to establish that the toxic effects have occurred; and (3) toxicity tests are necessary to establish that the adverse effects can be caused by the toxicity of the wastes. Even with this information, relationships between toxic wastes and ecological effects may be difficult to determine. Comparisons of these three data types are greatly simplified when the data collection activities are coordinated. Space and time coordination of data collection is necessary to eliminate variation in the analytical results associated with the difference in geographical regions and changes in concentration and toxicity over time.

Due to the complexities inherent in statistical sampling design, this chapter will not attempt to present specific field sampling designs appropriate for an HWS. The following discussion focuses on general approaches and issues in field sampling design.

### 4.1.1 Theoretical Considerations

The ecological assessment will draw on both laboratory and field data. Most of the field data will be observational data, or what Hurlburt (1984) terms results from mensurative experiments. Generally, different methods are used to analyze data from field studies than laboratory studies, primarily because most field data are not generated by randomized controlled experiments. This has the following two major implications: (1) many commonly used statistical analysis techniques, e.g., analysis of variance (ANOVA), or hypothesis tests, are not applicable or are restricted in interpretation; and (2) inferences of causality are usually not possible from observational field data alone.

It is worthwhile to review the essentials of classical experimental design to appreciate these two points. Consider the simplest case, where one wishes to determine if a particular treatment has an effect. A target population of subjects is identified, and two groups are selected at random from the target population. The treatment is administered to one group, and the other group serves as the control. A response is measured for each group, and the difference in the average response is a measure of the effect of the treatment. The significance of the difference can be established by standard hypothesis tests. Moreover, the random assignment of subjects to treatment and control groups permits an inference of causality: one can claim that the observed difference is in fact due to the treatment and not to some preexisting difference between the groups.

In an ecological assessment, the treatment and control groups are not selected at random from some target population, since, in fact, the HWS site was not selected at random. No amount of careful matching of a reference area outside the HWS can compensate for the lack of random selection. A statistically valid test of the hypothesis that any observed difference between the HWS and the reference site is due to the HWS is not possible. One can test, however, the hypothesis that the two sites are different, but that difference cannot be attributed to the presence of the HWS. In statistical terms, the effect of the HWS is completely confounded with preexisting differences between the HWS and a reference site.

This does not mean that a firm case cannot be made that an HWS has had an adverse ecological effect. However, in doing so, it must be recognized that the HWS itself represents an experimental unit that cannot be replicated. Some care must be exercised to avoid "pseudoreplication" (Hurlbert 1984). In essence, pseudoreplication is testing an hypothesis about treatment effects with inappropriate statistical design or analysis methods. It is as much a problem of misspecification or misunderstanding of the hypothesis being tested as of methodological errors. For the case at hand, pseudoreplication can be avoided by recognizing that the hypothesis of an effect of the HWS cannot be tested by statistical means. The hypothesis of a difference between a reference site and the HWS can be tested. Of course, establishing a difference is an essential step in the process of demonstrating an adverse ecological effect. If there is no detectable difference, then there is no cause to establish. Non-statistical methods must be used to establish that the difference is caused by the presence of the HWS.

Methods used to establish causality may make use of statistical techniques, such as regression or correlation. For example, regression can be used to show that toxicity increases along with the concentration of some chemical known to originate from the HWS. The regression merely describes the relationship, there is no implication of a causal link. The presence of a strong relationship is evidence that the link exists.

### 4.1.2 Practical Considerations

A major step in assessing ecological effect at an HWS will be the choice of a reference site for comparison. The case for causality can be strengthened by selecting the reference site to be as similar as possible to the HWS. In making the selection, physical similarities (e.g., elevation, landscape shape, soils), environmental similarities (e.g., precipitation, temperature, wind patterns, external sources of pollution), and ecological similarities (e.g., habitat type, habitat disturbance) should all be considered. If the site is aquatic, then parameters such as stream order, flow rate, and stream hydrograph should be considered. Additional references on site selection are presented in Chapters 6 through 8.

Every effort should be made to ensure that the samples are collected, stored, and processed under a uniform protocol. The same volume or weight should be collected and the samples should be stored in identical containers. The samples should be processed as soon as possible, and the time between collection and processing should be as uniform as possible.

A guiding principle is that one should avoid the possibility of creating a handling effect that is confounded with an effect being measured. If delays in sample processing are unavoidable, the samples should be processed either in a random order or with a balanced intermixture of treatment and controls. If more than one field team is to be used, the sample locations assigned to a team should be distributed randomly over the site.

The field technicians should have explicit, detailed instructions on the sampling protocol. The instructions should include not only the actual sample collection procedure, but also details of sample site location. Since the sample sites will likely be located at random, occasionally there will be some sites selected that cannot be sampled. For example, the presence of a large boulder just below ground surface may preclude soil sampling. Contingency procedures should be established to cover such events.

## 4.2 SAMPLE DESIGN DEVELOPMENT

The most important consideration in the design of any sampling plan is a clear, precise statement of the objective of the sampling (see Chapter 3). This should include a statement of the general question that is to be addressed, along with specific working hypotheses that can be used to guide the design development, description of the specific endpoints to be assessed, and specification of the measurements to be made and the data to be collected. Potential questions that might influence the design of a sampling plan include: "What are the effects on terrestrial or aquatic organisms; what is the severity of maximum effects; and what is the spatial distribution of effects?" Because a unified sampling approach is essential, all anticipated measurements should be considered before attempting to design the sampling plan. Chemical concentrations must relate to observed effects, so it would not make sense to sample once to determine spatial distribution of chemical concentration, and to make a second sample to determine distribution of ecological effects. Eventually, measures of intensity of insult will be tied to measures of effect, and the most direct means of accomplishing that is to have all samples taken at the same location. All available information should be considered in designing the sampling plan.

The sample design will be largely determined by the measurement endpoints. The selection of such endpoints should be made early in the design process, and the design built around that selection. Statistical consideration should be given to the selection of endpoints. From a statistical standpoint, a good endpoint should have the following two properties: (1) a low natural variability, and (2) a monotonic response that is steep relative to the natural variability. Natural variability contributes to the standard error of any statistic (e.g., a mean or a regression coefficient) computed from the data. Lower natural variability permits reliable inferences with smaller sample sizes.

Data analysis techniques that will be used directly affect the sample design, and vice versa. Different sample designs are optimal for estimating LC50 isopleths than for estimating the average LC50.

## 4.3 SELECTION OF SAMPLE DESIGN

The selection of an appropriate sample design is dependent upon a number of variables such as the objective of the study, prior knowledge of the physical and chemical characteristics of the HWS, the data analysis technique of interest, and the degree of sensitivity necessary to validate the study. This section will review a number of candidate sampling design methods. Additional information can be found in Bratcher (1970), Cochran (1977), and Green (1979).

### 4.3.1 Terminology

The sampling design process begins with definition of the target population. In statistical terminology, the basic entity that is to be measured is called a population element. In many cases, elements are selected for measurement in groups, called sampling units. In field sampling, the collection of points that comprise a particular area might be considered the population elements. For sampling purposes, the area might be divided into subregions, such as quadrats. The quadrats would then be the sample units.

Once the sampling units have been identified, they must be arranged, at least conceptually, in some manner so that they become available for sampling. Such an arrangement is called a population frame (Cochran 1977). Construction of the population frame is frequently one of the more challenging aspects of constructing a good sample. Conceptually, there are numerous ways to arrange sample points. A frequently used method is to arrange the points in a grid pattern, with the points equidistant in an X-Y coordinate system. An alternative method is to arrange the points along a transect, with the sample points equidistant along a straight line. The sample points may be chosen randomly within the area of interest. Each of these methods is discussed further below.

### 4.3.2 Non-Random Methods

A number of techniques are available for selecting particular sample locations. A frequently used method in field sampling is to select sites based on scientific judgment. For instance, sites may be selected that are thought to be representative or typical based on the preliminary survey; or presumably-sensitive sites may be chosen. Such judgmental selection may sometimes be the best way of estimating an average or detecting an effect. However, a serious flaw of such methods is that the quality is highly dependent upon the skill of the person making the selection. The estimates may be very good and very accurate, but there is no means to assess their goodness or accuracy.

A second method is to locate the sample sites in a regular pattern, either at the nodes of a grid or at regular locations along a transect. This method has the advantages of good spatial coverage and greater objectivity. There are, however, two major disadvantages: a regular sample spacing may miss a periodic pattern; and again, there is no inherent means of assessing the precision of the sample.

### 4.3.3 Random Methods

Statistical theory provides a means of evaluating precision only if the sample selection is random. In simple random sampling, every sampling unit in the population frame has the same chance of being included in the sample. Simple random sampling is conveniently used with a list frame where the entire target population can be enumerated. With the sampling units numbered sequentially, selection can be done with the aid of a random number table or with computer-generated random numbers. Simple random sampling has the advantage of objectivity as well as several important statistical advantages. First, most statistics (e.g., means and regression coefficients) generated from the sample data are unbiased estimates of the corresponding parameters of the whole sample region. Second, the statistical analysis of data from points located completely at random is comparatively straightforward. Finally, and most important, the method provides built-in estimates of precision. Some drawbacks are that completely random sampling may miss important characteristics of the site, spatial coverage tends to be non-uniform, and many points may be in areas of little interest.

### 4.3.4 Stratified Sampling

Some of the difficulties mentioned above may be partially overcome through the use of stratified sampling. Stratification consists of dividing the target population into several groups, or strata, and then selecting independent samples from each stratum. Stratified sampling is most often used to increase precision by sampling more intensively the more variable portions of a target population. However, it can also be used to allocate more sampling effort to important subpopulations without losing the ability to make entire population projections. For instance, it may be prudent to sample regions of known or suspected high chemical concentrations more intensively than regions of lower concentration.

The techniques discussed in the preceding paragraph can be combined in a variety of ways to incorporate the best features of each. A good sample design has at least the following features: (1) samples are located so that they provide the maximum amount of information about the site; (2) sample points have a uniform spatial distribution; and (3) an internal method for estimating precision is available as an adjunct to the design.

If the preliminary survey has provided a rough indication of the regions of interest, then the sample should be allocated so that critical regions are well characterized. Once that is done, then points within an identified subregion should be located according to a regular grid pattern. In order to preserve the randomness essential for estimates of precision, the grid should be oriented at random on the site. This can be accomplished by locating two points at random, and positioning the grid so that both points lie on a grid line and the first point lies on an intersection of grid lines. The coordinates of the points selected at random should be chosen using a table or computer-generated list of random numbers.

## 4.4 DETERMINATION OF SAMPLE SIZE

One of the first questions often asked of a statistician is: "How many samples should I take?" Unfortunately, there is no simple and strictly correct answer. Generally speaking, the precision of an estimate, whether it be an estimate of a mean or an estimate of the slope of a regression line, is expressed in terms of a standard error. The standard error is determined by four factors: inherent population variability, sample size, sampling design, and the data analysis method. In principle, one can determine a sample size by deciding on the required precision and using the known relationships between standard error, sample size, population distribution, and analysis method. However, the exact relationships are usually complex and depend on unknown population characteristics such as the population variance. Thus, some approximate guidelines are usually applied. Other things being equal, the standard error will be roughly inversely proportional to the square root of sample size. Increasing the sample size from, for instance, 10 to 40, will double the precision (halve the uncertainty). A further reduction by a factor of 0.5 would require a sample size of 160. The gain in precision for smaller samples will be relatively rapid.

A second consideration in selecting sample size is the balance between Type I errors (rejecting a true null hypothesis) and Type II errors (accepting a false null hypothesis). Consider the comparison of a reference site to the HWS by a test of significance for a difference between the two, and suppose that an adverse effect corresponds to a decrease in the average. The null hypothesis is that the mean response at the HWS is the same as the mean response at the reference (REF) site ($H_0$:$m_{HWS} = m_{REF}$), and the alternative is that the mean response at the HWS is less than (or greater than) the mean response at the reference site ($H_A$: $m_{HWS} < m_{REF}$). The Type I error rate, i.e., the significance level of the hypothesis test, is controlled by specifying the minimum observed difference between $m_{HWS}$ and $m_{REF}$ that will lead to rejection of $H_0$. The Type II error rate is frequently expressed in terms of the power of a test, which is the probability of falsely accepting the null hypothesis. The power is determined by the test method, the significance level, the sample size, the sampling method, and the population variance. In an ecological assessment, the power is at least as important, and possibly more important, than the significance level. The consequences of rejecting the hypothesis of no effect when in fact there is an adverse effect may be more severe, economically and socially, than the consequences of remediation on a site that may not have needed it.

Most of the statistical tests used in the assessment of an HWS will involve comparisons of two sample means: one from the HWS and one from a reference site. Determination of sample size requires the specification of test method, the power, the significance level, and magnitude of the difference to be detected. For purposes of illustration, suppose that the means are to be compared using a t-test. If the value of the population standard deviation, s, is known (not estimated from the data), the necessary sample can be calculated from the following formula:

$$n = 2(Z_a + Z_b)^2(s/d)^2$$

where:

$n$ = sample size
$Z_a$ = normal score corresponding to the significance level
$Z_b$ = normal score corresponding to the Type II error
$d$ = size of the difference to be detected
$s$ = population standard deviation.

For ease in calculation of sample size, the values of $(Z_a + Z_b)^2$ are given in Table 4-1 for various values of the significance level and the power for a one-tailed test.

**Table 4-1. Multipliers of $2(s/d)^2$ for Determination of Sample Size**

| Significance level | Power | | | |
|---|---|---|---|---|
| | .75 | .8 | .9 | .95 |
| .2 | 2.3 | 2.8 | 4.5 | 6.2 |
| .1 | 3.8 | 4.5 | 6.6 | 8.6 |
| .05 | 5.4 | 6.2 | 8.6 | 10.8 |
| .01 | 9.0 | 10.0 | 13.0 | 15.8 |

For example, suppose the population standard deviation is known to be 7.5, and a difference of 10 or larger is deemed to be important. Further, suppose a 90% chance of detecting that difference at a 5% significance level is needed. The required sample size is calculated as follows:

$$n = (2)(8.6)(7.5/10)^2 = 9.675, \text{ rounded up to } 10.$$

This method should be used only if the population standard deviation is known and not estimated from the data. If the standard deviation must be estimated from the data, the sample size should be inflated accordingly. An approximate adjustment can be made by first calculating the sample size as above, and then multiplying by a factor of $(n+3)/(n+1)$. In the example above, if 7.5 were an estimate instead of a known population standard deviation, the appropriate sample size would be

$$n' = 9.675(10+3)/(10+1) = 11.43, \text{ rounded up to } 12.$$

Another important consideration in picking a sample size is that statistical methods for "large" samples tend to be much simpler than for small samples. Although the dividing line between large and small is not firm, a sample size of 30 to 50 is generally sufficient to use large sample methods. A sample size of ten should be treated as a small sample.

## 4.5 REFERENCES

Bratcher, T.L., M.A. Moran, and W.J. Zimmer. 1970. Tables of sample size in the analysis of variance. Pages 156-164. In: Journal of Quality Technology.

Cochran, W.G. 1977. Sampling Techniques. John Wiley & Sons. New York, NY.

Green, R.H. 1979. Sampling Design and Statistical Methods for Environmental Biologists. John Wiley and Sons, New York, NY. 257 pp.

Hurlburt, S.H. 1984. Pseudoreplication and the design of ecological field experiments. Ecological Monographs. 54:187-211.

# CHAPTER 5

# QUALITY ASSURANCE AND DATA QUALITY OBJECTIVES

By

William Warren-Hicks, Kilkelly Environmental Associates, Raleigh, NC

## 5.1 QUALITY ASSURANCE

Agency policies require that all EPA laboratories, program offices, and regional offices participate in a well managed quality assurance (QA) program when environmental data is collected. This policy extends to those monitoring and measurement efforts supported or mandated through contracts, regulations, and/or other formal agreements. The intent is to develop a unified approach to QA to ensure the collection of data that are scientifically sound, legally defensible, and of known quality.

Quality assurance practices include all aspects of laboratory and field procedures that affect the accuracy and precision of the data, such as sample handling and storage, condition of monitoring equipment, field and laboratory conditions, record keeping, and data evaluation. The importance of QA in the ecological assessment of a hazardous waste site (HWS) cannot be over stressed. A QA plan should be developed for all data generating activities associated with ecological assessments at HWSs (U.S. EPA 1987).

Specific, formal QA procedures have been well defined for some disciplines (e.g., aquatic toxicity testing) and are under development in other disciplines (e.g., vertebrate field surveys). Due to this inconsistency, applicable QA recommendations and references have been included within the individual sections of this manual. For those sections with little QA information, the reader should refer to the Quality Assurance Guidelines for Biological Testing (U.S. EPA 1978).

## 5.2 DATA QUALITY OBJECTIVES (DQOs)

Environmental data play a critical role in the ecological assessments of HWSs. Due to the importance of data collection in the decision making process, the methods used to design data collection programs should place substantial emphasis on defining the regulatory objectives of the program, the decision that will be made with the data collected, and the possible consequences of an incorrect decision. A design process that fails to explore these issues and focuses only on collecting the "best possible data" can result in serious problems. Data collection programs based on technical merit alone do not always ensure that adequate information is obtained from a decision-making perspective.

This chapter provides a brief overview of the role of data quality objectives (DQOs) in the design of data collection programs. For a more thorough discussion see U.S. EPA 1987a and 1987b.

### 5.2.1 Overview of DQOs and the DQO Process

The Quality Assurance Management Staff (QAMS) has proposed an approach to designing environmental data collection programs based on the development of DQOs. The DQO process does not use a pre-established budget as the sole constraint on the design of a data collection program. Rather, equal consideration is given to defining the quality of the product needed, i.e., the degree to which total error in the results derived from data must be controlled to achieve an acceptable level of confidence in a decision that will be made with the data. The DQO process provides a logical, objective, and quantitative framework for finding an appropriate balance between the time and resources that will be used to collect data and the quality of the data needed to make the decision. Therefore, data collection programs based on DQOs may be more likely to meet the needs of decision makers in a cost effective manner.

DQOs are statements of the level of uncertainty that a decision maker is willing to accept in results derived from environmental data, when the results are going to be used in a regulatory or programmatic decision (e.g., defining that a new regulation is needed, setting or revising a standard, or determining compliance). To be complete, these quantitative DQOs must be accompanied by clear statements of the following:

- the decision to be made,
- why environmental data are needed and how they will be used,
- time and resource constraints on data collection,
- descriptions of the environmental data to be collected,
- specifications regarding the domain of the decision, and
- the calculations, statistical or otherwise, that will be performed using the data in order to arrive at a result.

Developing DQOs should be the first step in initiating any significant environmental data collection program that will be conducted by or for the EPA. The DQO process consists of three stages with several steps in each stage (Figure 5-1). The first two stages result in proposed DQOs, with accompanying specifications and constraints for designing the data collection program. In the third stage, potential designs for the data collection program are evaluated. The following section provides a brief overview of the three stages.

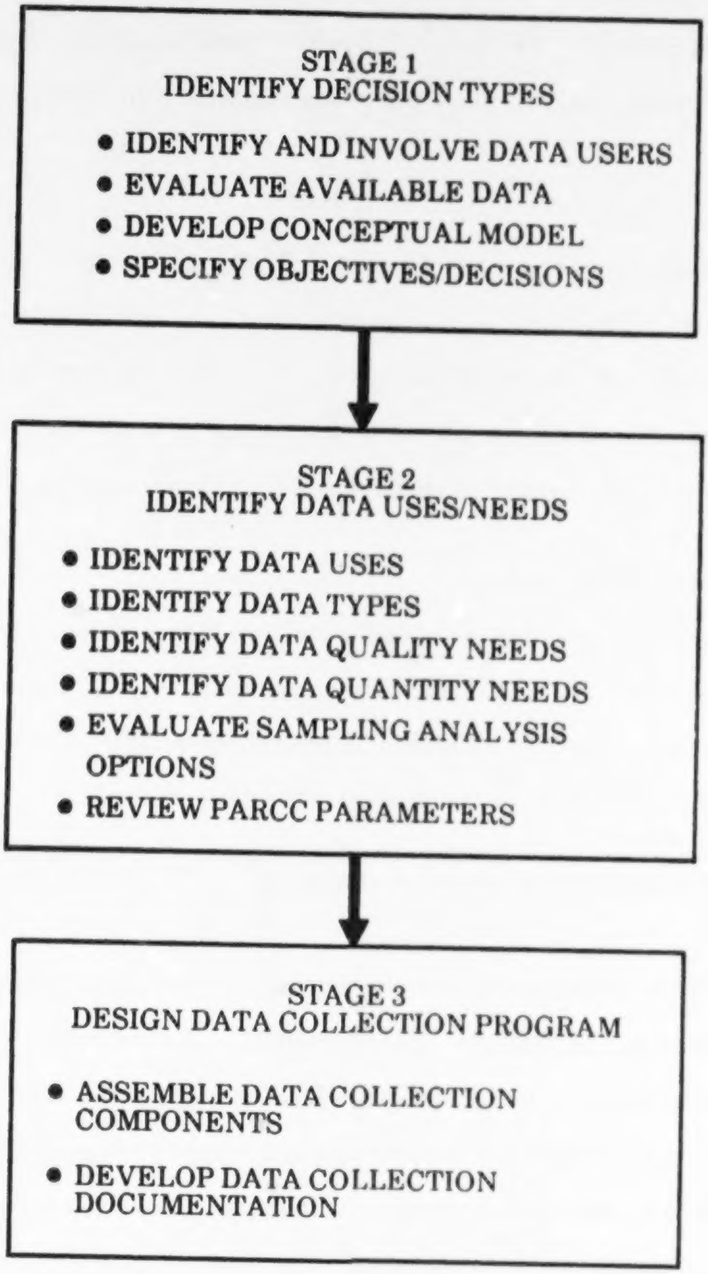
STAGE 1
IDENTIFY DECISION TYPES
• IDENTIFY AND INVOLVE DATA USERS
• EVALUATE AVAILABLE DATA
• DEVELOP CONCEPTUAL MODEL
• SPECIFY OBJECTIVES/DECISIONS
STAGE 2
IDENTIFY DATA USES/NEEDS
• IDENTIFY DATA USES
• IDENTIFY DATA TYPES
• IDENTIFY DATA QUALITY NEEDS
• IDENTIFY DATA QUANTITY NEEDS
• EVALUATE SAMPLING ANALYSIS OPTIONS
• REVIEW PARCC PARAMETERS
STAGE 3
DESIGN DATA COLLECTION PROGRAM
• ASSEMBLE DATA COLLECTION COMPONENTS
• DEVELOP DATA COLLECTION DOCUMENTATION

Figure 5-1. The DQO three-stage process.

### 5.2.2 The Three Stages of the DQO Process

The following discussion presents a brief overview of the three stages within the DQO development process.

#### 5.2.2.1 Identify Decision Types

Stage I is the responsibility of the decision maker. The decision maker states an initial perception of what decision must be made, what information is needed, why and when it is needed, how it will be used, and what the consequences will be if information of adequate quality is not available. Initial estimates of the time and resources that can reasonably be made available for the data collection activity are presented.

#### 5.2.2.2 Identify Data Uses and Needs

Stage II is primarily the responsibility of the senior program staff, with guidance and oversight from the decision maker and input from the technical staff. The information from Stage I is carefully examined and discussed with the decision maker to ensure that senior program staff understand as many of the nuances of the program as possible. After this interactive process, senior program staff discuss each aspect of the initial problem, exercising their prerogative to reconsider key elements from a technical or policy standpoint. The outcome of their work, once explained and concurred upon by the decision maker, leads to the generation of specific guidance for designing the data collection program. The products of Stage II include proposed statements of the type and quality of environmental data required to support the decision, along with other technical constraints on the data collection activity that will place bounds on the search for an acceptable design in Stage III. These outputs are proposed DQOs.

### 5.2.2.3 Design the Data Collection Program

The responsibility of the technical staff and the decision maker during Stage III is to assure the outputs from Stages I and II are understood. The objective of Stage III is to develop data collection plans that will meet the criteria and constraints established in Stages I and II. All viable options should be presented to the decision maker. It is the prerogative of the decision maker to select the final design that provides the best balance between time and resources available for data collection and the level of uncertainty expected in the final results.

## 5.3 REFERENCES

United States Environmental Protection Agency. 1978 Environmental Monitoring Series. Quality assurance guidelines for biological testing. EPA/600/4-78/043. Environmental Monitoring Support Laboratory, Las Vegas, NV.

United States Environmental Protection Agency. 1987. Quality Assurance Program Plan. Environmental Research Laboratory, Corvallis OR.

# CHAPTER 6

# TOXICITY TESTS

By

**Benjamin R. Parkhurst, Western Aquatics, Inc., Laramie, WY.**

**Greg Linder, NSI Technology Services Corporation,**
**Corvallis Environmental Research Laboratory, Corvallis, OR.**

**Karen McBee, Department of Zoology,**
**Oklahoma State University, Stillwater, OK**

**Gabriel Bitton, Department of Environmental Engineering Sciences,**
**University of Florida, Gainesville, FL.**

**Bernard J. Dutka, Canada Center for Inland Waters,**
**Burlington, Ontario, Canada.**

**Charles W. Hendricks, U.S. Environmental Protection Agency,**
**Corvallis Environmental Research Laboratory, Corvallis, OR.**

## 6.1 GENERAL OVERVIEW OF TOXICITY TESTS -- Benjamin R. Parkhurst and Greg Linder

### 6.1.1 Introduction

Toxicity to aquatic and terrestrial organisms including microbial populations is a potential concern at hazardous waste sites. Toxicity tests, when combined with chemical analyses, may show that adverse effects were caused by toxic chemicals originating from the hazardous waste site. This information, used in conjunction with field surveys which show that adverse ecological effects have occurred, can be used to establish a link between hazardous wastes and adverse ecological responses. Without field and laboratory data, other potential causes of the observed effects, such as habitat alteration or natural variability, which are not directly related to the toxic effects of the hazardous wastes, cannot be eliminated.

This chapter reviews the application of environmental toxicology to hazardous waste site evaluations. This information would be used to help assess the potential role of toxic hazardous wastes in causing adverse ecological effects.

### 6.1.2 Alternative Approaches to Assessing Toxicity

The toxicity of environmental media potentially contaminated by hazardous wastes can be estimated using two approaches: a toxicity-based approach or a chemistry-based approach. In the toxicity-based approach, toxicity tests directly measure toxic effects. Toxicity testing involves the measurement of a biological effect (e.g., death) associated with exposure to complex mixtures in instances when the mechanisms of the observed effect are not readily apparent and the specific causes of the effect are often unknown. The toxicity-based approach was developed for measuring and regulating the toxicity of complex effluents discharged to surface waters (U.S. EPA 1985). It has also been used to identify and characterize toxic wastes under Resource Conservation and Recovery Act (RCRA) regulations (Millemann and Parkhurst 1980) and the Superfund Acts (Greene et al. 1988).

In the chemistry-based approach, chemical analyses and laboratory-generated water quality (or air, soil, or sediment) criteria are used to estimate toxicity. For example, if concentrations of specific chemicals in surface waters (or air, soil, or sediment) exceed criteria values, then the concentrations are considered to be toxic. The chemistry-based approach is also used for regulating waste water discharges under the Clean Water Act and to characterize toxic wastes under RCRA and Superfund.

Rationale for using the toxicity-based approach include:

- Water and air quality criteria are available for relatively few chemicals potentially present in hazardous wastes. Soil and sediment quality criteria are not yet available for any chemicals.
- Water, air, soil, and sediment quality criteria do not account for additive, synergistic, or antagonistic interactions among toxic chemicals in a complex mixture.
- Toxicity tests measure the aggregate toxicity of all constituents in a complex mixture, including additive, synergistic, and antagonistic effects.
- Chemical analyses for complex mixtures (many chemicals present), especially for organics, can be more expensive than toxicity testing.
- The specific chemicals analyzed in complex mixtures may not include many toxic chemicals actually present.
- It is not always clear from chemical data which compounds are causing toxicity in a complex hazardous waste mixture.
- The bioavailability of toxic chemicals is evaluated with toxicity tests but not with chemical analyses; therefore, chemical data may over- or under-estimate the toxicities of single chemicals.

The chemistry-based approach may be appropriate for:

- Simple mixtures (few chemicals present), where chemical analyses can be less expensive than toxicity testing;
- Specific problem chemicals, such as carcinogens or bioaccumulative chemicals, which can be directly measured; and
- Designing treatment systems, which are more easily designed to remove specific chemicals than to reduce a generic parameter such as toxicity.

Both of these approaches complement each other, and depending on site-specific conditions, either or both may be appropriate for assessing the toxicity of environmental media contaminated by hazardous wastes. However, it is now generally considered that for complex chemical mixtures of unknown composition, such as hazardous waste site samples, the toxicity-based approach is better for estimating potential toxicity (Bergman et al. 1986; U.S. EPA 1985; U.S. FWS 1987; Greene et al. 1988).

### 6.1.3 Toxicity Data

Toxicity tests can provide data on the acute (short-term) and chronic (long-term) toxicity of contaminated media to aquatic and terrestrial biota. These tests are generally conducted using standard, laboratory test species; but in some cases, tests on resident species may be appropriate. If the test species are representative of sensitive, resident species, the toxicity data may provide an assessment of the potential for causing the adverse effects measured in field surveys.

Toxicity tests are generally run in toxicology laboratories on samples collected at the site. Most tests are static or static-renewal tests. Flow-through aquatic or atmospheric tests may also be conducted on-site in a mobile laboratory; alternatively, in situ toxicity tests, can be done to provide realistic, continuous exposures to ambient concentrations of hazardous wastes. For in situ toxicity tests, test organisms are exposed on site by placing them into containers, establishing and monitoring vegetation plots, marking and then recapturing animals or a similar approach. The test species can be either standard laboratory or resident species.

Three types of endpoints are derived from the acute and chronic toxicity tests: (1) percent survival of the test organisms in 100% site sample (water, soil, or sediment) in laboratory tests or in situ exposures; (2) a concentration-percent survival relationship for laboratory tests run at several test concentrations of the surface water, soil, or sediment; and (3) estimates of LC50s (e.g. mortality), EC50s (e.g. growth and reproduction), MATCs, etc. Methods for analyzing these different types of toxicity data are discussed by Peltier and Weber (1985), Horning and Weber

(1985), Rand and Petrocelli (1985), Dixon et al. (1985), Finney (1978), and Montgomery and Peck (1982).

The survival data for 100% test concentrations and the in situ exposure data provide information on the direct toxicity of ambient concentrations of hazardous waste chemicals. These data can be directly compared to survey data to assess probable sources and causes of toxic effects. For example, if a 100% concentration of the test material in a laboratory (or in situ) exposure caused mortality to fathead minnows, and the fish community of the site is affected, then there is a high probability that toxicity is causing the adverse effects. The concentration-percent survival relationship could be used to extrapolate the toxicity data to sites with decreasing concentrations of the hazardous waste materials. The LC50 and MATC estimates are most useful for comparisons of toxicity among different samples or sites.

Acute tests measure lethal effects, but sublethal effects (e.g., behavior) can also be measured. Acute toxicity test results are usually expressed as LC50s (the concentration of a chemical or mixture in the exposure medium which is estimated to be lethal to 50% of the test organisms), EC50s (the concentration of a chemical or mixture in the exposure medium that is estimated to have a sublethal effect to 50% of the test organisms), or LD50s (the dose of a chemical or mixture in the organism that is estimated to be lethal to 50% of the test organisms) for the test duration. For example, the 96-hour LC50 is the estimated concentration that will kill 50% of the test organisms in 96 hours of exposure. Other effect levels besides 50%, (e.g., the LC1) can be estimated. Concentration versus effect relationships can be determined by analyzing the data using various regression techniques (Finney 1978; Montgomery and Peck 1982; Dixon et al. 1985).

LC50s are generally used in reference to aquatic toxicity test results in which exposure is measured as the concentration of the toxic material. LC50s are also used in reference to terrestrial toxicity test results with atmospheric gases and soils. LD50s are generally used in reference to laboratory toxicity tests with chemicals that are ingested or assimilated by animals or plants. In such tests, exposure is measured as the dose of the chemical the organism receives.

Chronic tests potentially detect both chronic lethal and sublethal toxicity, such as effects on growth and reproduction. Chronic test results can be expressed in the same manner as acute test results, but they are often expressed as estimates of acceptable concentrations or toxicity threshold concentrations. For example, the MATC (maximum acceptable toxicant concentration) is usually presented as two test concentrations. One, the NOEC (no-observed-effects-concentration), is the highest test concentration that caused no statistically significant toxic effects. The NOEC is an estimate of an acceptable concentration. The second, the LOEC (lowest-observed-effects-concentration), is the lowest concentration that caused statistically significant toxic effects. These two values, the NOEC and LOEC, span the toxicity threshold for the chemical. The GMATC (geometric mean of the MATC, i.e., the NOEC and LOEC) is an estimate of the chronic toxicity threshold. Peltier and Weber (1985) and Horning and Weber (1985) provide detailed discussions of these toxicity values and methods for their calculation.

### 6.1.4 Integration of Toxicity Tests with Field Surveys

Field surveys can identify adversely affected communities and can provide information for assessing adverse ecological effects potentially caused by hazardous wastes. However, field surveys alone can not identify causes of effects. Toxicity tests in conjunction with appropriate chemical data can establish potential causes. The actual causes may be hazardous wastes, but effects could also be caused by habitat degradation, external sources of toxic chemicals, natural variability, etc.

In general, toxicity data and field survey results should be integrated using, for example, exploratory data analysis. These preliminary analyses should be considered part of the site assessment, but the relationships between the toxicity-derived and field-derived data sets will be correlative and suggest cause-effect relationships. Possible cause and effect relationships can be supported by chemical analyses. In complex mixtures, however, it may be impossible to determine which chemical or chemicals are causing toxicity. Various fractionation and toxicity identification techniques are used to more completely evaluate the causative toxic chemicals in complex mixtures (Parkhurst 1986; U.S. EPA 1985; U.S. EPA 1988).

### 6.1.5 State of the Science

The state of the science for environmental toxicology is reviewed briefly below. The discussion is largely based on aquatic toxicology, since this area is generally more developed than others. However, most of the discussion should be relevant to other areas of environmental toxicology.

#### 6.1.5.1 Test Species

Toxicity tests that are used to identify probable sources and causes of toxic effects at hazardous waste sites should use species representative of the ecosystem being assessed. It is not necessary to use test species from the ecosystem in question, as long as the species used are representative of the ecosystem. Sensitivities of aquatic biota to toxic chemicals vary widely among species. Sensitivities vary less within taxa (i.e., among species of the same genera) and within similar classes of chemicals such as non-pesticide organics, pesticides, inorganics, and metals (LeBlanc 1984;

Slooff et al. 1986). Kenaga (1979) reported that, given the LC50 for a particular chemical and species, relatively reliable LC50s can be calculated (through the use of empirically derived equations) for the effect of the same chemical on other species.

LeBlanc (1984) found that algae, invertebrates, and fish responded similarly to non-pesticide organics, but the sensitivities of fish and invertebrates to pesticides were not highly correlated. A high correlation was determined in sensitivities of fish and invertebrates to metals, but the degree of sensitivity varied by an order of magnitude. These studies indicate that the comparative sensitivities of aquatic organisms depend on their phyletic relationships and on the type of chemical (Slooff et al. 1986).

### 6.1.5.2 Use of Acute Toxicity Data to Predict Chronic Toxicity

It appears that for similar classes of chemicals and similar taxa, acute-to-chronic ratios established for one species and chemical can be used to estimate the chronic toxicity of the chemical to another species. Such extrapolations should only be made for the same types of tests conducted under the same conditions (e.g., water quality, life stage).

Kenaga (1979) reported that the LC50 is not useful for predictions of chronic toxicity. However, Slooff et al. (1986) found that the uncertainty in predicting chronic toxicity from acute toxicity data for a given species is smaller than the uncertainty in predicting acute toxicity between species. The U.S. EPA (1986) makes extensive use of species acute-to-chronic ratios in the derivation of water quality criteria for toxic chemicals.

### 6.1.5.3 Use of Short-Term Tests to Predict Chronic Toxicity

Several short-term tests have been designed to estimate chronic toxicities. Tests such as the 7-day *Ceriodaphnia* sp., 7-day fathead minnow, 21-day *D. magna* tests, and 30 to 90 day fish early life stage (ELS) tests, are widely used to predict the chronic toxicities of chemicals and mixtures (Mount and Norberg 1984, 1985; Rand and Petrocelli 1985; McKim 1985; Urban and Cook 1986; ASTM 1988). Life-stage sensitivities vary greatly within species. Fry and larvae are often the most sensitive stages for fish, while eggs are relatively resistant. Beyond the fry or larval stage, sensitivity often decreases as size increases. Consequently, in full life cycle exposures, the sensitivity of early life stages will largely determine the sensitivity of the species to the chemical. Thus, ELS tests generally provide good estimates of the effects of full life cycle chronic exposures (McKim 1977, 1985; Macek and Sleight 1977). Kenaga (1979) also found that MATCs derived from critical life stages (usually eggs and fry) of fish appear to be good substitutes for MATCs derived from complete life cycle toxicity tests. These tests are generally considered to provide good estimates of chronic toxicity endpoints in much less time and at much less cost than full life cycle tests. Consequently, more materials and species can be tested. Field validation studies have supported the validity of using these short-term tests to predict population- and community-level effects *in situ* (U.S. EPA 1985).

### 6.1.5.4 Extrapolation of Laboratory Results to Predict *In Situ* Toxicity

Laboratory acute and chronic tests appear to be reasonable models of toxicity in receiving waters under similar exposure conditions (U.S. EPA 1985). Parkhurst (1987) found that laboratory test results could provide good estimates of *in situ* toxicity for the same species, if the laboratory test conditions (e.g., water quality, test species strain and size) closely simulated *in situ* conditions. The degree of correlation is directly related to the amount of similarity between laboratory and field conditions. Laboratory tests may be conservative estimators of in situ toxicity because in nature many chemicals degrade, transform, complex, precipitate, or adsorb, which reduces their bioavailability (Kimerle et al. 1986).

### 6.1.5.5 Use of Single-Species Test Results to Predict Population, Community, and Ecosystem Effects

A concern frequently raised in the use of single-species toxicity tests is that these tests fail to measure higher-level ecological effects, such as effects on interspecies interactions, ecosystem structure, and ecosystem function (Cairns 1985). Consequently, assessments based on single-species toxicity tests may not adequately predict ecosystem-level effects.

However, from the standpoint of assessing causes of adverse ecological effects, it is not critical that single-species tests measure effects on ecosystem structure and function. What is important is that assessments based on single-species tests identify the probable sources and causes of toxic effects to ecosystem structure and function. It is presently unknown whether interactions between species within a community are more sensitive than the most sensitive component species (Mount 1985). However, since all biological functions within an ecosystem are carried out by specific organisms, community sensitivity should only be an expression of individual species sensitivity. Thus, any function within an ecosystem should not be more sensitive than the species that perform those functions. For single-species tests to be used to adequately predict the probable sources and causes of these community functions requires the use of adequately sensitive single-species tests.

Slooff's (1985) analysis of data for 38 compounds indicates that concentrations that are acutely toxic to single species are usually not much greater than concentrations that are toxic at the ecosystem level. Whereas, concentrations that are toxic in chronic single-species tests are, in most cases, overprotective of ecosystems. These results imply that single-species tests have a certain predictive capability for higher-order response levels.

At present, it appears that assessments of sources and causes of adverse ecological effects based on toxicity tests with representative, sensitive, single species should be adequate to identify causes of toxicity at the population, community, and ecosystem level. If anything, assessments based on single-species test results appear to be conservative estimators of higher-level effects. While work to date generally suggests that assessments based on single-species tests will not lead to false negatives, more field evaluations are necessary to support the hypotheses regarding the robust characteristics of toxicity assessments.

#### 6.1.5.6 Multi-Species Toxicity Tests

Multi-species tests are defined as tests that include more than one species in the test chamber (Cairns 1985). Definitions and classifications for different types of multi-species tests are not standardized. Multi-species tests include tests with two species, such as predator-prey and competition tests; model ecosystems such as microcosms, mesocosms, macrocosms, limnocorrals, and artificial streams; and field studies in natural surface water bodies. Good reviews of multi-species test methods can be found in Hammons (1981) and Cairns (1985).

Use of multi-species tests as research tools is widely accepted, but their use in impact assessments has been limited, since it remains unclear whether such tests will improve the results of the assessments. Historically, support for multi-species tests in ecological assessments of toxic effects has been based, in part, on their hypothesized greater sensitivity than single-species tests. There is no consensus, however, that multi-species tests are more sensitive than the individual species that comprise those test systems. Because nearly all community functions can be adequately performed by numerous species, the most important reason to use multi-species tests may be that single-species tests are likely to be too sensitive. Multi-species tests seem to be more important when undisturbed function and structure is the goal, rather than, for example, when a sport fishery for an introduced species is the goal (Mount 1985).

Microcosms and other model ecosystem tests have received limited use in toxicity assessments, and their applicability appears to be much narrower. Multi-species tests may be best suited for supplying information on a site- or subregion-specific basis (Kooijman 1985).

Since, in the overall ecological assessment process, aquatic field surveys are used to assess ecological effects, multi-species tests are not necessary to test for higher-level ecological effects. A battery of sensitive single-species tests is adequate for identifying sources and probable causes of toxicity at hazardous waste sites.

### 6.1.6 References

American Society for Testing and Materials (ASTM). 1988. 1988 Annual Book of Standards. Section 11, Water and Water Engineering, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA.

Bergman, H.L., R.A. Kimerle and A.W. Maki, eds. 1986. Environmental Hazard Assessment of Effluents. Pergamon Press, Elmsford, NY.

Cairns, J., Jr., ed. 1985. Multispecies Toxicity Testing. Pergamon Press, Elmsford, NY.

Dixon, W.J., M.B. Brown, L. Engelman, J.W. Frane, M.A. Hill, R.I. Jennrich, and J.D. Toporek. 1985. BMDP Statistical Software. University of California Press, Berkeley, CA. 734 pp.

Finney, D.J. 1978. Statistical Method in Biological Assay, Third Edition. Charles Griffin and Company, Ltd., London.

Greene, J.C., W.J. Warren-Hicks, B.R. Parkhurst, G.L. Linder, C.L. Bartels, S.A. Peterson, and W.E. Miller. 1988. Protocols for Acute Toxicity Screening of Hazardous Waste Sites, Final Draft. U.S. Environmental Protection Agency, Corvallis, OR. 145 pp.

Hammons, A.S., ed. 1981. Ecotoxicological Test Systems: Proceedings of a Series of Workshops. EPA/560/6-8/-004. Office of Toxic Substances, U.S. Environmental Protection Agency, Washington, DC.

Horning, W.B., II, and C.I. Weber. 1985. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. EPA/600/4-85/014. Environmental monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH.

Kenaga, E.E. 1979. Aquatic test organisms and methods useful for assessment of chronic toxicity of chemicals. Pages 101-111. In: Dickson, K.L., A.W. Maki and J. Cairns, Jr., eds. Analyzing the Hazard Evaluation Process. Water Quality Section, American Fisheries Society, Bethesda, MD.

Kimerle, R.A., W.J. Adams and D.R. Grothe. 1986. A tiered approach to aquatic safety assessment of effluents. Pages 247- 264. In: H.L. Bergman, R.A. Kimerle and A.W. Maki, eds. Environmental Hazard Assessment of Effluents. Pergamon Press, Elmsford, NY.

Kooijman, S.A.L.M. 1985. Toxicity at population level. Pages 143-164. In: J. Cairns, Jr., ed. Multispecies Toxicity Testing. Pergamon Press, Elmsford, NY.

LeBlanc, G.A. 1984. Interspecies relationships in acute toxicity of chemicals to aquatic organisms. Environmental Toxicology and Chemistry. 3:47-60.

Macek, K.J. and B.H. Sleight. 1977. Utility of toxicity tests with embryos and fry of fish in evaluating hazards associated with the chronic toxicity of chemicals to fishes. Pages 137-146. In: F.L. Mayer and J.L. Hamelink, eds. Aquatic Toxicology and Hazard Evaluation. ASTM STP 634. American Society for Testing and Materials, Philadelphia, PA.

McKim, J.M. 1977. Evaluation of tests with early life stages of fish for predicting long-term toxicity. J. Fish Res. Board Can. 34:1148-1154.

McKim, J.M. 1985. Early life stage toxicity tests. Pages 58-95. In: Rand, G.M. and S.R. Petrocelli, eds. Fundamentals of Aquatic Toxicology: Methods and Applications. Hemisphere Publishing Corp., New York, NY.

Milleman, R.E. and B.R. Parkhurst. 1980. Comparative toxicity of solid waste leachates to Daphnia magna. Environ. Internat. 4:255-260.

Montgomery, D.E. and E.A. Peck. 1982. Introduction to Linear Regression. John Wiley and Sons, New York, NY. 504 pp.

Mount, D.I. 1985. Scientific problems in using multispecies toxicity tests for regulatory purposes. Pages 13-18. In: J. Cairns, Jr., ed. Multispecies Toxicity Testing. Pergamon Press, Elmsford, NY.

Mount, D.I. and T.J. Norberg. 1984. A seven-day life cycle cladoceran toxicity test. Env. Tox. Chem. 3:425-434.

Mount, D.I. and T.J. Norberg. 1985. A new subchronic fathead minnow (Pimephales promelas) toxicity test. U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN.

Parkhurst, B.R. 1986. The role of fractionation in hazard assessments of complex effluents. In: H.L. Bergman, R.A. Kimerle and A.W. Maki, eds. Environmental Hazard Assessment of Effluents. Pergamon Press, Elmsford, NY.

Parkhurst, B.R. 1987. A comparison of laboratory and in situ bioassays for evaluating the toxicity of acidic waters to brook trout. Ph.D. Dissertation, University of Wyoming, Laramie, WY.

Peltier, W. and C.I. Weber. 1985. Methods for Measuring the Acute Toxicity of Effluents to Aquatic Organisms. Third Edition. EPA/600/4-85/013. Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH.

Rand, G.M. and S.R. Petrocelli, eds. 1985. Fundamentals of Aquatic Toxicology: Methods and Applications. Hemisphere Publishing Corp., New York, NY.

Slooff, W. 1985. The role of multispecies testing in aquatic toxicology. Pages 45-60. In: J. Cairns, Jr., ed. Multispecies Toxicity Testing. Pergamon Press, Elmsford, NY.

Slooff, W., J.A.M. van Oers and D. de Zwart. 1986. Margins of uncertainty in ecotoxicological hazard assessment. Environmental Toxicology and Chemistry. 5:841-852.

U.S. Environmental Protection Agency. 1985. Technical support document for water quality-based toxics control. Office of Water, U.S. Environmental Protection Agency, Washington, DC.

U.S. Environmental Protection Agency. 1986. Quality criteria for water 1986. EPA/440/5-86/001. Office of Water Regulations and Standards, U.S. Environmental Protection Agency, Washington, DC.

U.S. Environmental Protection Agency. 1988. Methods for aquatic toxicity identification evaluations. Phase I: Toxicity characterization procedures (Draft) Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC.

U.S. Department of Interior. 1987. Type B technical information, Injury to Fish and Wildlife Species, CERCLA Project 301. Washington, DC.

Urban, D.J and N.J. Cook. 1986. Hazard evaluation division standard evaluation procedure: Ecological risk assessment. EPA/540/9-85/001. Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington, DC.

## 6.2 AQUATIC TOXICITY TESTS -- Benjamin R. Parkhurst

### 6.2.1 Introduction

Aquatic toxicology has been widely used to assess toxic effects of complex chemical mixtures to aquatic ecosystems (Bergman et al. 1986). Development of standardized, consensus methods for aquatic toxicity testing is more advanced than other areas of environmental toxicology. Most tests developed for testing complex mixtures are directly applicable to hazardous waste site testing, with few modifications. A sufficient number of standardized, "off-the-shelf" tests are presently available to fill most testing needs for ecological assessments of hazardous waste sites.

### 6.2.2 Aquatic Toxicity Test Methods

The methods available for hazardous waste site assessments are grouped into two categories: (1) Class I methods are off-the-shelf techniques that are widely accepted and ready for general use; and (2) Class II methods are less widely used, or being developed as applied methods pertinent to toxicity assessments for HWSs.

To meet the goal of yielding the most information on a cost-effective basis and being easily interpreted by decision makers, toxicity tests used in hazardous waste site assessments should use standardized, generally accepted methods that can be performed with a reasonable amount of time, money, effort, and expertise. Many aquatic toxicity tests have been standardized, and tests are presently available to meet most testing needs for hazardous waste site assessments. The sediment toxicity tests discussed in this chapter, although not yet standardized, are in widespread use and are considered applicable for general use.

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### 6.2.2.1 Test Species

Species used in aquatic toxicity tests may include virtually any species that can be maintained in laboratory (or in situ) exposure chambers. However, as discussed in section 6.2.3.3, it is usually not necessary to conduct tests on resident species. The tests recommended in the following subsections use primarily standard laboratory test species.

### 6.2.2.2 Dilution Water

Of special concern is the source and quality of dilution water used in toxicity tests. Two options are available: (1) use site dilution water, collected upstream of the potential source of hazardous waste toxicity; or (2) use a reconstituted dilution water, which is similar to on-site water in respect to pH, hardness, alkalinity, and salinity (Peltier and Weber 1985; Weber et al. 1988). Choice of method will depend on site-specific considerations. It is generally preferable to use site dilution water; however, if this water is toxic, it may not be usable; alternatively, the toxicity of the dilution water can be factored into the analysis of the toxicity of the test material (U.S. EPA 1985).

### 6.2.2.3 Laboratory and QA/QC Requirements

Peltier and Weber (1985), Horning and Weber (1985), and Weber et al. (1988) provide detailed descriptions of laboratory and QA/QC requirements for aquatic toxicity testing. Virtually all tests can be run in either on-site or off-site laboratories.

### 6.2.2.4 Class I Methods

**6.2.2.4.1** Acute Toxicity Methods. Many acute toxicity test methods have been developed for both single chemical and complex mixture testing (OECD 1984; U.S. EPA 1978a-b, 1982a-c, 1985; Peltier and Weber 1985; Rand and Petrocelli 1985;

ASTM 1988; Greene et al. 1988). Acute test methods directly applicable to hazardous waste site assessments are those used for whole effluent testing and whole sediment testing.

(A) Acute Toxicity Methods: Aqueous Samples. Standardized, consensus methods for conducting acute aquatic toxicity tests are available for a large number of marine and freshwater fish, invertebrates, and plants. Inter- and intra-laboratory comparisons have demonstrated that the reproducibility of standardized toxicity tests can be as good as routine chemical analyses (U.S. EPA 1985). The following three tests are recommended.

(1) Peltier and Weber (1985). This manual describes flow-through, static-renewal, and static methods for measuring the acute toxicity of effluents to a wide variety of freshwater and marine fish as well as invertebrates. Static-renewal or static procedures are generally used to test hazardous waste sites. ASTM (1988) also describes similar methods for acute toxicity testing of effluents and surface waters.

(2) Greene et al. (1988). This manual describes short-term methods specifically designed for measuring the toxicity of solid and aqueous samples from hazardous waste sites to Daphnia magna, D. pulex, algae (Selenastrum capricornutum), and fathead minnows (Pimephales promelas). The toxicities of solid samples to aquatic species are tested by preparing elutriates (see section 6.3) for testing. Except for the preparation of the elutriates, these methods are similar to Peltier and Weber (1985).

(3) ASTM (1988). This manual describes a method for conducting static acute toxicity tests with larvae of four species of marine bivalve mollusks, which are not included in Peltier and Weber (1985).

(B) Acute Toxicity Methods: Sediment Samples. No standardized, consensus sediment toxicity tests are yet available. However, several test methods are in widespread use and are undergoing standardization by ASTM. In addition, the tests listed in subsection 6.2.2.4.1 (A) are applicable to sediment testing with minor modifications (see ASTM 1988).

6.2.2.4.2 Chronic Toxicity Methods. Chronic tests are, by definition, of longer term than acute tests; but to be useful in the decision making process for hazardous waste site assessments, information on toxicity must be obtained in a relatively short time. Relatively few standardized, consensus methods are presently available for doing chronic toxicity tests, primarily due to a lack of knowledge for culturing many species through complete life cycles in the laboratory. A lack of knowledge of the basic biology of many present and potential test species impedes the use of additional species (Loewengart and Maki 1985). However, the reproducibility of chronic toxicity tests can also be good (Parkhurst et al. 1981; U.S. EPA 1985).

Chronic toxicity tests that are of long duration will have less utility in assessing the effects of hazardous waste sites than tests of short duration. In recent years, there has been considerable effort devoted by the EPA and others to develop short-term tests that accurately estimate the chronic toxicity of effluents and receiving waters. These tests, recommended below, are directly applicable for hazardous waste site evaluations.

(A) Chronic Toxicity Methods: Aqueous Samples.

(1) Horning and Weber (1985). This manual describes four short-term tests useful for estimating the chronic toxicity of waters contaminated by hazardous wastes to three freshwater species: (1) the alga, Selenastrum capricornutum; (2) fathead minnows; and (3) Ceriodaphnia dubia. These procedures are presently applied to test the chronic toxicities of a wide variety of effluents and should be applicable to most hazardous waste site assessments.

(2) Weber et al. (1988). This manual describes marine and estuarine tests, analogous to the freshwater tests described above, for sheepshead minnow (Cyprinodon variegatus), inland silverside (Menidia beryllina), the mysid (Mysidopsis bahia), the sea urchin (Arbacia punctulata), and the alga (Champia parvula).

(3) ASTM (1988). The ASTM 1988 Annual Book of Standards describes life-cycle toxicity tests for Daphnia magna and saltwater mysids, and early life stage tests for a variety of fish species. These tests are of longer duration (21 to 120 days, depending on the species) than those described above. They may be desirable for answering questions of special interest at some hazardous waste sites.

(B) Chronic Toxicity Methods: Sediment Samples. No standardized, consensus methods for chronic toxicity testing of sediments are yet available.

### 6.2.2.5 Class II Methods

6.2.2.5.1 Acute Toxicity Methods: Aqueous Samples. Although acute, aquatic toxicity test methods are continually being refined and improved, the test methods listed in section 6.2.2.4.1 (A) above are sufficient to conduct hazardous waste site assessments at this time.

In situ toxicity tests are an alternative testing procedure that would provide realistic, continuous exposures to ambient concentrations of hazardous waste chemicals at lower cost than with a mobile laboratory. Test organisms (e.g., fish) are placed in cages in site waters to test toxicity in situ (Johnson et al. 1987; Parkhurst 1987). These tests are relatively simple to perform, but the methods lack standardization.

**6.2.2.5.2 Acute Toxicity Methods: Sediment Samples.** Acute, sediment toxicity tests are under development, but are currently restricted to macroinvertebrates for both freshwater and marine testing. Standardization of several methods is under way by ASTM. However, some methods (freshwater midge, freshwater and marine amphipods), have undergone some standardization and are in sufficiently widespread use to be considered ready for general use. Currently, the draft ASTM methods are recommended for sediment toxicity tests for freshwater and marine sediments. (Copies of these drafts may be obtained by contacting the chair of ASTM subcommittee E-47.03 for Sediment Toxicology at ASTM Headquarters in Philadelphia, PA).

**6.2.2.5.3 Chronic Toxicity Methods: Aqueous Samples.** Many chronic tests methods are potentially available for hazardous waste site assessments (see Rand and Petrocelli 1985), but most are of too long duration for practical use. Several standardized chronic toxicity test methods are under development by ASTM Committee E-47; however, the methods listed in section 6.2.2.4.2 (B) should be adequate for doing chronic toxicity assessments at most hazardous waste sites.

6.2.2.5.4 Chronic Toxicity Methods: Sediment Samples. No standardized or consensus chronic sediment toxicity tests are yet available for either freshwater or marine testing. However, some non-standardized chronic sediment tests are available (see Swartz 1987 for a review of test methods).

### 6.2.3 Methods Integration

The sequential approach outlined below is one of many available to those who use these methods and may suggest appropriate toxicity tests for hazardous waste site evaluations and for integrating methods. The approach consists of the following steps: (1) identify surface waters; (2) assess adverse ecological effects; (3) conduct acute toxicity tests; (4) evaluate acute toxicity; (5) conduct chronic toxicity tests; and (6) evaluate chronic toxicity. These steps are discussed in the following subsections.

#### 6.2.3.1 Identify Surface Waters

For each candidate site for an ecological assessment, identify all surface waters that potentially contain aquatic biota. If surface waters are not present or if, because of habitat or flow limitations, they can not support a significant aquatic community, then there is no need for aquatic toxicity testing. If surface waters are present and they sustain or could sustain an aquatic community potentially affected by hazardous wastes, then toxicity testing is appropriate to assess the probable sources and causes of adverse ecological effects.

#### 6.2.3.2 Assess Adverse Ecological Effects

The aquatic field survey methods described in section 8.2 provide the data necessary to assess adverse ecological effects potentially caused by hazardous wastes. The survey identifies specific, adversely affected aquatic communities and the extent of the effect. At this point, the actual cause of those impacts are unknown, but may include toxic hazardous wastes.

### 6.2.3.3 Conduct Acute Toxicity Tests

If adversely affected aquatic communities are identified, conduct acute toxicity tests on potentially contaminated surface water and sediment samples, using a battery of tests and test species, including species representative of each community. If adversely affected communities are not found, testing may be desirable to confirm the lack of toxicity.

As noted in section 6.2.2.1, species selected as test organisms do not have to include resident species, but should include those standard, laboratory test species that are taxonomically, ecologically, and/or physiologically most similar to resident species. For example, *Daphnia spp*. could be surrogates for resident zooplankton, *Selenastrum capricornutum* could be a surrogate for resident algae, fathead minnows could be surrogates for resident warmwater fish, *Lemna minor* could be a surrogate for resident aquatic macrophytes, etc. It may not be necessary to conduct tests for surrogates of communities for which no ecological effects were identified in the aquatic surveys. For example, if aquatic macrophytes communities are not adversely affected, it may not be necessary to do aquatic macrophyte toxicity tests. Again, if adversely affected communities are not apparent, testing may still be desired to confirm the lack of toxicity.

### 6.2.3.4 Evaluate Acute Toxicity

The acute toxicity test results provide quantitative information on the direct toxicity of ambient concentrations of hazardous waste chemicals. These data can be directly compared to aquatic survey data to assess probable sources and causes of toxic effects.

For example, if 100% solution causes mortality to fathead minnows in the laboratory or in situ, and the fish community of the site is adversely affected, then there is a high probability that toxicity is causing the effect. The concentration-percent survival relationship could be used to extrapolate the toxicity data to downstream sites with decreasing concentrations of the hazardous waste solutions. The LC50 data would be most useful for comparisons of acute toxicity among different samples or sites.

#### 6.2.3.5 Conduct Chronic Toxicity Tests

If no acute toxicity is detected, but adverse ecological effects are apparent, then chronic toxicity tests should be run. Chronic tests may also be run to confirm the presence or absence of toxicity, regardless of the presence of adverse ecological effects. Refer to section 6.2.2.4 for guidance on selection of tests to run.

#### 6.2.3.6 Evaluate Chronic Toxicity

Chronic tests potentially detect both chronic lethal and sublethal toxicity, such as effects on growth or reproduction. These data are used to assess probable causes and sources of adverse ecological effects in the same manner as for acute toxicity data. Methods for analyzing and interpreting chronic toxicity data are provided in Chapter 9.

### 6.2.4 Case Studies

A series of studies conducted by the EPA have established that the results of ambient toxicity tests are generally significantly correlated with effects to periphyton, zooplankton, benthic macroinvertebrates and fish (Mount et al. 1984; Mount and Norberg-King 1985; Mount et al. 1986a; Mount et al. 1986b; Norberg-King and Mount 1986; Mount et al. 1986c; Mount and Norberg-King 1986).

### 6.2.5 References

American Society for Testing and Materials (ASTM). 1988. 1988 Annual Book of Standards. Section 11, Water and Water Engineering, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA.

Bergman, H.L., R.A. Kimerle and A.W. Maki, eds. 1986. Environmental Hazard Assessment of Effluents. Pergamon Press, Elmsford, NY.

Greene, J.C., W.J. Warren-Hicks, B.R. Parkhurst, G.L. Linder, C.L. Bartels, S.A. Peterson, and W.E. Miller. 1988. Protocols for Acute Toxicity Screening of Hazardous Waste Sites, Final Draft. U.S. Environmental Protection Agency, Corvallis, OR.

Horning, W.B., II, and C.I. Weber. 1985. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. EPA/600/4-85/014. Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH.

Johnson, D.W., H.A. Simonin, J.R. Colquhoun, and F.R. Flack. 1987. In situ toxicity of fishes in acid waters. Biogeochemistry. 3:181-208.

Loewengart, G. and A.W. Maki. 1985. Multispecies toxicity tests in the safety assessment of chemicals: Necessity or curiosity? Pages 1-12. In: J. Cairns, Jr., ed. Multispecies Toxicity Testing. Pergamon Press, Elmsford, NY.

Mount, D.I., N. Thomas, M. Barbour, T. Norberg, T. Roush, and R. Brandes. 1984. Effluent and ambient toxicity testing and instream community response on the Ottawa River, Lima, Ohio. EPA/600/2-84/080. Permits Division, Office of research and Development, Duluth, MN.

Mount, D.I. and T.J. Norberg-King, eds. 1985. Validity of effluent and ambient toxicity tests for predicting biological impact, Scippor Creek, Circleville, Ohio. EPA/600-3085/044. U.S. Environmental Protection Agency.

Mount, D.I. and T. Norberg-King. 1986. Validity of effluent and ambient toxicity tests for predicting biological impact, Kanawha River, Charleston, West Virginia. EPA/600/3-86/006. U.S. Environmental Protection Agency.

Mount, D.I., A.E. Steen, and T. Norberg-King. 1986a. Validity of effluent and ambient toxicity tests for predicting biological impact, Back River, Baltimore Harbor, Maryland. EPA/600/8-86/001. U.S. Environmental Protection Agency.

Mount, D.I. , T. Norberg-King, and A.E. Steen. 1986b. Validity of effluent and ambient toxicity tests for predicting biological impact, Naugatuck River, Waterbury, Connecticut. EPA/600/8-86/005. U.S. Environmental Protection Agency.

Mount, D.I. , A.E. Steen, and T. Norberg-King. 1986c. Validity of effluent and ambient toxicity tests for predicting biological impact, Ohio River, Wheeling, West Virginia. EPA/600/3-85/071. U.S. Environmental Protection Agency.

Norberg-King, T. and D.I. Mount. 1986. Validity of effluent and ambient toxicity tests for predicting biological impact, Skeleton Creek, Enid, Oklahoma. EPA/600-3085/044. U.S. Environmental Protection Agency.

Organization of Economic Cooperation and Development (OECD). 1984. Guidelines for testing chemicals. Section 4: Health effects. Director of Information, OECD, 2, Rue Andre-Pascal, 75775 Paris CEDEX 16, France.

Parkhurst, B.R. 1987. A comparison of laboratory and *in situ* bioassays for evaluating the toxicity of acidic waters to brook trout. Ph.D. Dissertation, University of Wyoming, Laramie, WY.

Parkhurst, B.R., J.L. Forte and G.P. Wright. 1981. Reproducibility of a life cycle toxicity test with *Daphnia magna*. Bulletin of Environmental Contamination and Toxicology. 26:1-8.

Peltier, W. and C.I. Weber. 1985. Methods for Measuring the Acute Toxicity of Effluents to Aquatic Organisms. Third Edition. EPA/600/4-85/013. Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH.

Rand, G.M. and S.R. Petrocelli, eds. 1985. Fundamentals of Aquatic Toxicology: Methods and Applications. Hemisphere Publishing Corp., New York, NY.

Swartz, R.C. 1987. Toxicological methods for determining the effects of contaminated sediments on marine organisms, Chapter 14. In: Dickson, K.L., A.L. Maki and W.A. Brungs, eds. Fate and Effects of Sediment-Bound Chemicals in Aquatic Systems. Pergamon Press, New York, NY.

U.S. Environmental Protection Agency. 1978a. Directory of short term tests for health and ecological effects. EPA/600/1-78/052. U.S. Environmental Protection Agency, Washington, DC.

U.S. Environmental Protection Agency. 1978b. Short-term tests for health and ecological effects. Part 1: Program overview and Part 2: Directory of tests. EPA/600/9-78/037. Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC.

U.S. Environmental Protection Agency. 1982a. Environmental effects test guidelines. EPA/560/6-82/002. U.S. Environmental Protection Agency, Washington, DC.

U.S. Environmental Protection Agency. 1982b. Pesticide assessment guidelines. EPA/540/9-82/018 through 028. Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington, DC.

U.S. Environmental Protection Agency. 1982c. Toxic substances test guidelines. EPA/6-82-001 through 003. Office of Toxic Substances, U.S. Environmental Protection Agency, Washington, DC.

U.S. Environmental Protection Agency. 1985. Technical support document for water quality-based toxics control. Office of Water, U.S. Environmental Protection Agency, Washington, DC.

Weber, C.I., W.I. Horning, D.J. Klemm, T.W. Neiheisel, P.A. Lewis, E.L. Robinson, J. Menkedick, and F. Kessler. 1988. Short-term methods for estimating the chronic toxicity of efluents and receiving waters to marine and estuarine organisms. (Draft)

EPA/600/4-87/028. Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH.

## 6.3 TERRESTRIAL TOXICITY TESTS -- Greg Linder and Karen McBee

### 6.3.1 Introduction

Terrestrial toxicity tests for soils and sediments from hazardous waste sites are less developed than aquatic toxicity tests (Fava et al. 1987). Although few terrestrial test methods have been standardized (OECD 1984), methods-standardization efforts have been initiated by the U.S. EPA (Greene et al. 1988a). The laboratory toxicity tests discussed in this section evaluate both the direct (e.g., soils and sediments) and indirect (e.g., laboratory-derived eluates from soils) toxicity of soil or sediment samples.

### 6.3.2 Terrestrial Toxicity Test Methods

### 6.3.2.1 Class I Methods

The toxicity tests summarized below represent a battery of Class I, single-species bioassays that have been used in toxicity assessments for hazardous waste site-soil and sediment samples (see Figure 6-1). For the most part, they are short-term tests for assessing the acute toxicity of soils or sediments. Standardized tests for assessing chronic toxicity are currently unavailable except for an algal toxicity test included in the terrestrial test battery. Complete listings of laboratory facilities and test requirements for Class I tests are found in Greene et al. (1988a). Summary outlines of these terrestrial toxicity tests follow. For additional information, consult Greene et al. (1988b), Peltier and Weber (1985), and Horning and Weber (1985).

6.3.2.1.1 Soil and Sediment Preparations. Soil and sediment samples from hazardous waste sites are heterogeneous mixtures of natural chemicals in the substrate matrix (e.g., clays and silts, and sands in varying proportions) (Bohn et al. 1979; Brady 1974), along with anthropogenic chemicals that may be present as contaminants (Morrill et al. 1982). Field sampling of soils and sediments is the most

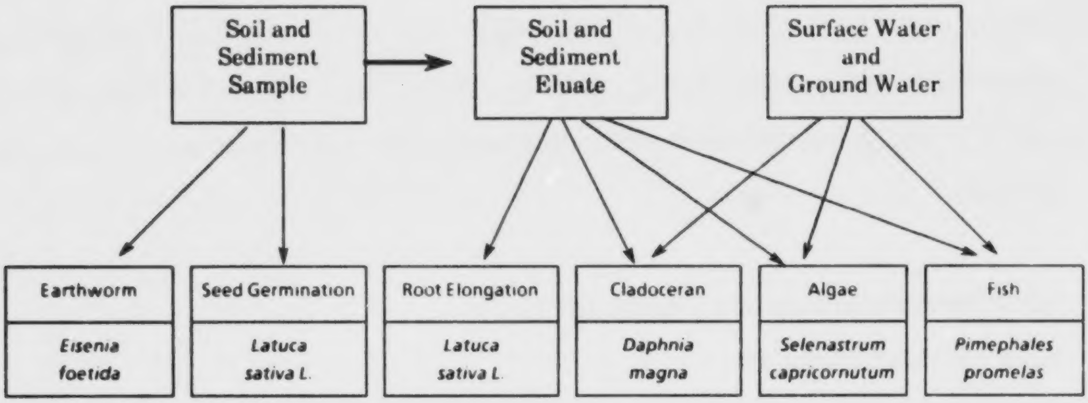
Soil and Sediment Sample
Soil and Sediment Eluate
Surface Water and Ground Water
Earthworm
*Eisenia foetida*
Seed Germination
*Latuca sativa L.*
Root Elongation
*Latuca sativa L.*
Cladoceran
*Daphnia magna*
Algae
*Selenastrum capricornutum*
Fish
*Pimephales promelas*

**Figure 6-1. Battery of single-species bioassays for various types of environmental samples.**

critical step in any terrestrial toxicity assessment, but particularly for those assessments that derive toxicity estimates from samples sent to off-site laboratories. Transit times and storage conditions during shipment potentially confound toxicity estimates generated by laboratories located great distances from the site itself. Depending upon site-specific considerations, soil and sediment samples should be taken at the same sites and times as chemical samples.

Earthworm and seed germination tests (see Figure 6-1) require the site sample to be screened through a 1/4" soil sieve prior to testing. The samples are mixed with artificial soil to produce a series of test soil concentrations. Greene et al. (1988a) should be consulted for complete details on sample preparation, testing, and data analysis.

6.3.2.1.2 Eluate Preparations from Site Soils and Sediments. Eluates are prepared from untreated site soils and sediments to evaluate the mobility of chemical constituents in hazardous wastes. Site samples are mixed with four milliliters of deionized water per gram (dry weight) soil or sediment. The slurry is then mixed in total darkness for 48 hours at 20° ± 2°C. After mixing, the resulting eluate is centrifuged and then filtered through a 0.45 µm cellulose acetate or glass fiber filter. Original sample moisture is incorporated into the eluate sample during its preparation. Hence, a constant "solute/solvent" ratio is assured during the extraction of any site sample.

6.3.2.1.3 Terrestrial Bioassays Performed on Site Soils and Sediments. Brief outlines of test procedures are presented in groups according to the type of sample being analyzed, as follows: (1) direct measures of soil and sediment toxicity derived from terrestrial bioassays, including a 14-day earthworm test and a 5-day seed germination test; and (2) indirect measures of toxicity derived using aquatic and terrestrial test systems, including a 4-day *Selenastrum capricornutum* test, the 2-day daphnid (*Daphnia magna* or *Daphnia pulex*) and fathead minnow (*Pimephales promelas*) tests, and the 5-day root elongation test.

(A) Eisenia foetida (Earthworm) 14-day Soil Acute Toxicity Test. Earthworms improve soil aeration, drainage, and fertility within terrestrial environments (Edwards and Lofty 1972) and are considered representative soil macroinvertebrates. The test represents a modification of a method developed by Goats and Edwards (1982). *Eisenia foetida* is used in these tests since it is easily cultured in the laboratory, reaches maturity in 7 to 8 weeks at 25°C, and is responsive to a wide range of toxicants. Earthworms are exposed to toxicant solubles in soil moisture and by direct contact with or ingestion of chemicals adsorbed on soil (Callahan et al. 1985).

Test soil concentrations should include a range of site soil or sediment concentrations (e.g., 80%, 40%, 20%, 10%, 5% and 0% site-sample, dry weight site sample/total dry weight). Artificial soil used in these preparations consists of 10% sphagnum peat, 20% colloidal kaolinite clay, and 70% grade-70 silica sand by weight. The site sample is incorporated into the artificial soil to yield a homogeneous exposure medium with the desired site soil or sediment concentrations. Soil moisture is adjusted to assure that the percent soil hydration is similar in all test concentrations. Once exposure systems are prepared, ten adult earthworms are added to three replicate chambers, and incubated at 20° ± 2°C for 14 days. Mortality is noted at the end of 14 days, and appropriate statistical techniques are applied to derive the LC50.

(B) Seed Germination Toxicity Test. This test measures the effects of hazardous wastes on seed germination, a critical stage in the developmental biology of plants. The test outlined in Greene et al. (1988a) represents a modification of the method of Thomas and Cline (1985). The primary test species is lettuce (Butter Crunch), Lactuca sativa L., although others can be used.

The test procedure involves grading the seeds and then preparing exposure systems using Petri dish bottoms and Ziploc bags. Treatments are set up to cover a range of test soil concentrations (e.g., 80%, 40%, 20%, 10%, 5%, and 0% site sample mixed with artificial soil). Test soils are loaded into Petri dish bottoms, and 40 seeds are planted per dish. After seeding, 16-mesh silica sand is layered over the seeds, and the Petri dish is irrigated to 85% water holding capacity. The Petri dish is then placed upright in a Ziploc bag and sealed, leaving as much air space as possible inside. The sealed bags are placed in a growth chamber for 120 hours (24° ± 2°C); the first 48 hours are completed in total darkness and the balance 16:8 hours light:dark. After 120 hours, the number of seeds that have germinated in each dish is determined by counting the number of seedlings that emerge above the soil surface. The LC50 is derived from statistical analysis on the count data at 120 hours.

### 6.3.2.1.4 Aquatic Bioassays Performed on Eluates.

(A) Selenastrum capricornutum Toxicity Test. The ecological significance of unicellular algae is widely recognized, particularly in regard to its function in primary production and oxygen evolution. Algal communities may be inhibited or stimulated by water quality changes.

The test involves adding algal cells to a series of concentrations of site surface water, groundwater or site soil/sediment eluate. The typical test yields an estimate of the EC50, as well as an evaluation of lethality. Following inoculation, test flasks are incubated for 96 hours at 24° ± 2°C and 4304 ± 430 lux (continuous). Cell counts, measured manually or by electronic particle counters, yield direct measures of algal biomass based upon cell counts and mean cell volumes. EC50s are estimated using appropriate statistical methods.

(B) Daphnia magna or D. pulex Toxicity Test. Soil and sediment eluates can be tested using either Daphnia magna or D. pulex. Species of choice is dependent upon the hardness of the sample being tested; for samples with hardness less than 80 mg/L only D. pulex should be used as test species.

The test uses neonates less than 24-hour old, which are exposed to test concentrations ranging from 100% to 0% site sample (control). The tests are conducted at 22° ± 2°C (16:8 hours, light:dark); replicates of 10 neonates each are placed into test chambers. Mortality is assessed at the end of the 48-hour exposure and the LC50 is calculated.

(C) Fathead Minnow Short-Term Toxicity Test. Fathead minnows (Pimephales promelas) are exposed for 48-hours to a logarithmic series of site-sample eluates; hence, the method (adapted from Peltier and Weber 1985; Horning and Weber 1985; and ASTM 1985) yields estimates of the acute toxicity of site-sample eluates.

Exposures are performed at 20° ± 2°C (16:8 L:D), and use ten, 3 to 5 day-old fathead minnows per test chamber. Mortality is measured at the termination of the test, and LC50s are calculated as percent site-sample.estimates (LC50s), expressed as percent site sample associated with 50% mortality.

(D) Root Elongation Toxicity Test. Root elongation is an important early developmental event in the growth and survival of plants. Unlike the seed germination test, the root elongation test evaluates only the water soluble constituents of a sample. As a general rule, root elongation is more sensitive than seed germination. This test may be done with a number of economically important species that germinate and grow rapidly, e.g., lettuce (butter crunch, Lactuca sativa L.).

The test is done with graded seeds, which are placed in Petri dishes. A logarithmic series of test concentrations plus controls (water samples, or soil or sediment eluates) is prepared and added to filter paper-lined Petri dish bottoms. The test solutions are absorbed by the filter paper in each Petri dish. The seeds are placed on the filter papers and incubated in a darkened, humid container at 24° ± 2°C for 120 hours. At the end of the test, root length is measured, and an estimate of the EC50 is calculated.

6.3.2.1.5 Quality Assurance/Quality Control. Quality assurance/quality control (QA/QC) measures must be specified prior to initiating toxicity assessments. Depending upon the site-specific DQOs, and the role that either laboratory or in situ toxicity tests share in the ecological assessment for the site, project personnel must delineate QA/QC guidelines appropriate to the assessment process. For laboratory toxicity tests, a minimum QA/QC program must include specifications for: (1) sampling and handling hazardous wastes; (2) the sources and culturing of test organisms; (3) instrument condition and calibration; (4) use of reference toxicants, adequate controls, and exposure replication; (5) recording keeping; and (6) data evaluation (see Horning and Weber 1985). QA/QC guidelines for Class I tests are found in Greene et al. (1988a).

### 6.3.2.2 Class II Methods

The methods discussed in the following sections are potential candidates for evaluating waste site toxicity either in the laboratory or field. For use in the field, in situ toxicity tests are being developed and evaluated; some in situ techniques have been applied to waste site evaluations to a limited extent (e.g., Rowley et al. 1983). In situ techniques applied on a site-specific basis may help integrate laboratory toxicity data with field-derived estimates of exposure, and subsequently yield an estimate of the hazard associated with a particular waste site.

Generally, in situ methods use resident species that naturally occur on or near a waste site, and can be captured to evaluate toxicity or exposure. Various levels of biological organization can be measured through in situ methods, ranging from cellular and molecular levels through population levels of organization. Depending upon the data quality objectives (DQOs, see Section 5) for the field assessment, the information gathered may yield either high or low resolution evaluations.

6.3.2.2.1 Chromosomal Aberration Assay. The chromosome aberration assay (CA) has been successfully used to assess genotoxic effects in mammals at four different hazardous waste sites (McBee 1985; McBee et al. 1987; Tice et al. 1987; Thompson et al. 1988) two of which are Superfund sites. This assay examines mitotic cells arrested at metaphase for alterations and/or rearrangements in the chromosomes. The occurrence of chromosomal aberrations correlates well with the presence of mutagens and is closely associated with carcinogenesis. This type of assay is widely used and accepted for in vivo analysis of clastogenic mutagens. Standardized protocols for assays conducted with laboratory species are available from several sources including Brusick (1980) and EPA (1985). These protocols have been successfully adapted for in situ use with several wild mammal species (Baker et al. 1982; McBee et al. 1987; Thompson et al. 1988) and should be readily adaptable to other species. Although background values for chromosome aberrations are available for a few species of wild mammals, it is still essential that studies at HWSs be designed to include concurrent chromosomal aberration analysis at carefully matched reference sites.

6.3.2.2.2 Terrestrial Vertebrate Acute and Subacute Toxicity Tests. Routine test methods (e.g., ASTM 1988; Buttler 1987; Cholakis et al. 1981; McCann et al. 1981; Schafer and Bowles 1985) that address chemical effects on avian and small mammal models have been developed in response to FIFRA and TSCA. Although only a few tests have been completed on hazardous waste site samples, the potential application of these methods to ecological assessments at hazardous waste sites can not be overlooked. For example, ASTM (1988) contains standard methods for conducting avian acute toxicity tests; on a site-specific basis, these methods may be amenable to hazardous waste site toxicity assessments. Similarly, ASTM (1985) contains standard practices for conducting acute toxicity tests with amphibians. EPA has produced toxicity test guidelines (1982a-c) for regulatory mandates other than hazardous wastes. Numerous short-term toxicity tests are now being developed that may be available for site evaluations (e.g., ASTM 1988); although they cannot be unequivocally endorsed, they deserve attention when DQOs and site-specific ecological assessments are being developed.

6.3.2.2.3 Terrestrial Invertebrate Toxicity Tests. Most terrestrial invertebrate toxicity test methods have been developed and used in regulatory programs other than hazardous waste site investigations. Most of these are laboratory tests with few (if any) field evaluations. Nonetheless, the methods warrant consideration since they may be useful in evaluating the ecological effects associated with hazardous waste sites. Candidate test methods include: (1) laboratory tests with crickets (Acheta deomesticus) (Walton 1980) or grasshoppers (Thomas et al. 1983) in either acute or short-term chronic testing formats; (2) in situ or laboratory toxicity tests with harvester ants (Pogonomyrmex spp.) (Gano et al. 1985); (3) in situ or laboratory toxicity tests with honey bees (Apis spp.) (Thomas et al. 1983, 1984; Bromenshenk 1985); and (4) laboratory tests with nematodes such as Caenorhbditis elegans (e.g., Popham and Webster 1979, 1982) or Panagrellus spp. (e.g., Samoiloff et al. 1980). Any of these tests may be valuable for site assessments, particularly in regard to longer-term effects (e.g., genotoxicity or mutagenicity). While the invertebrate species available for toxicity testing are relatively limited at present, critical species have been identified (Wilson et al. 1987) and should be considered during the development of DQOs for any particular site.

6.3.2.2.4 Short-Term Plant Toxicity Assessment Methods. Currently, standardized terrestrial toxicity tests for plants are limited. The most promising methods still requiring standardization and/or evaluation include the following: (1) Tradescantia toxicity tests (stamen hair mutagenicity assay and micronuclei formation) (e.g., Grant and Zura 1982; Lower et al. 1983; Ma and Harris 1985; Lower et al. 1988); (2) the hexaploid virescent wheat assay for detecting cytogenetic effects (Redei and Sandhu 1988; Lower et al. 1988); and (3) the soil fungi response (e.g., sclerotia formation) tests (Thomas et al. 1983). The Tradescantia toxicity tests offers the opportunity for integration of laboratory and field tests, especially when resident species can be used as in situ biological indicators. The hexaploid virescent wheat assay has been used primarily in laboratory settings for evaluating clastogenicity from exposure to single chemicals and multi-chemical mixtures. Soil fungi response testing has been used in site evaluations on a limited basis to assess formation in response to complex chemical mixtures. This type of testing may complement other Class I microbial tests.

### 6.3.3 Methods Integration

As summarized in Figure 6-2, hazard assessment considers toxicity and exposure functions implicit to site evaluations. Ecological assessments at hazardous waste sites can potentially contribute to estimates of exposure. Depending upon the toxicity assessment methods indicated by the site- specific DQOs, the field methods employed should, as a minimum requirement, yield samples that assure adequate toxicity estimates for the site.

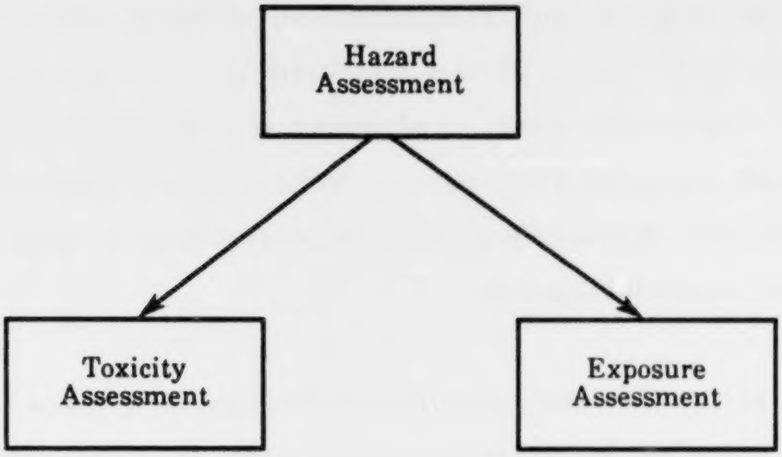

**Figure 6-2. Considerations in hazard assessment.**

A primary rationale for performing toxicity tests arises from the complexity of the systems being evaluated (Miller et al. 1985). The value of comparative toxicity data bases and the role of toxicity test batteries in site evaluation can be illustrated through case studies (also see Section 9). For example, Thomas, et al. (1986) used Class I tests for a toxicity assessment of Rocky Mountain Arsenal, near Denver, Colorado (see Table 6-1). The toxicity of soils from the site was evaluated, and the role of toxicity tests for site evaluations was demonstrated. For example, the test results distinguished between the toxicity from exposures to site soil (direct test systems) and that associated with exposures to water soluble soil contaminants (indirect test systems). Similarities and differences among endpoints for the two types of test systems were related to site-specific characteristics such as soil type and potential for groundwater contamination. Similarly, direct assessments of soil toxicity provided short-term measures of biological effects; Thomas, et al. (1986) analyzed these within comparative contexts as part of their evaluation of hazardous waste effects on soil biota. Although fewer terrestrial tests were conducted than aquatic tests, comparisons between direct estimates of soil toxicity (e.g., earthworm mortality and seed germination) also contributed to the site assessment for Rocky Mountain Arsenal. Again, different sensitivity and resistance patterns were evident from such a comparative approach.

In general, site-specific toxicity potentials may be suggested by comparing estimates of toxicity derived from indirect and direct test systems. These toxicity estimates will be of greater relevance when field surveys are completed in conjunction with toxicity tests. Additionally, interspecies variability and differences in biological responses become apparent in exposures to complex chemical mixtures and afford preliminary observations regarding contaminant characteristics. For example, on the bases of chemical analyses, site history, and known biological responses to single-compounds,

Table 6-1. **EC50 Response of Percent Inhibition Caused by Chemical Contaminants in Rocky Mountain Arsenal Soil Elutriate, Wastewater, and Ground Water Samples (modified after Thomas, et al. 1986)**

| Rocky Mountain Arsenal sample number | Major contaminants | Test | | | | |
|---|---|---|---|---|---|---|
| | | Algae[a] | Daphnia[a] | RE[b] | Seed germination | Earthworm |
| 085 | Heavy metals, pesticides | 8.3 | 86 | NE | --- | >25[c] |
| 092 | Heavy metals, pesticides | 6.4 | 25 | 61 | --- | <5.0 |
| F basin water | Heavy metal, DIMP, other organics | 0.002 | 0.003 | 1.0 | 0.5[d] | --- |
| F basin wellwater | DIMP, other organics | 27 | 21 | 12 | --- | --- |
| 1-5 | Unknown | S | 72 | 72[e] | 91[e] | 62 |
| 6 | Unknown | S | 94 | --- | 100 | 55 |
| 7 | Unknown | NE | NE | 32 | 100 | <25 |
| 8 | Unknown | S | NE | 19 | 92 | 58 |
| 9 | Unknown | S | NE | 26 | 13 | NE |

NE, no biologically significant toxicity observed; DIMP, diisopropylmethylphosphonate; S, growth stimulation.

[a] EC50, % elutriate or % water.

[b] RE, lettuce root elongation test; EC50 % elutriate or % water.

[c] Earthworm 14-d soil test LC50 values; % soil.

[d] LD50 value in % F basin water.

[e] 72/100 = 72% inhibition of lettuce root elongation in 100% soil elutriate or seed germination in 100% soil (seed germination). Seed germination results are the means of three replicates of 40 seeds each.

and complex mixtures, Thomas, et al. (1986) identified the most likely toxicants present in the complex mixture. Equally important, suspected toxicants were also eliminated on the basis of the toxicity expressed by different components of the test battery. Though toxicity assessments may show correlation between toxicity data and ecological effects, direct cause-effect relationships can only be inferred. This becomes even more relevant when complex chemical mixture exposures are evaluated, or multiple routes of exposure are assessed in the toxicity test.

### 6.3.4 References

American Society for Testing and Materials (ASTM). 1985. Standard practice for conducting acute toxicity tests on aqueous effluents with fishes, macroinvertebrates, and amphibians. ASTM Committee E-47, American Society for Testing and Materials, Philadelphia, PA.

American Society for Testing and Materials (ASTM). 1988. ASTM Book of Standards. Section 11, Water and Environmental Technology, Vol. 11.04. Pesticides; Resource Recovery; Hazardous Substances and Oil Spill Response; Waste Disposal; Biological Effects. American Society for Testing and Materials, Philadelphia, PA.

Baker, R.J., M.W. Haiduk, L.W. Robbins, A. Cadena, and B.F. Koop. 1982. Chromosomal studies of South American bats and their systematic implications. Special Publication. Pymatuning Laboratory. Ecol. 6:303-327.

Bohn, H.L., B.L. McNeal, and G.A. O'Connor. 1979. Soil chemistry. John Wiley & Sons, New York, NY.

Brady, N.C. 1974. The Nature and Properties of Soils. MacMillan Publishing Co., Inc., New York, NY.

Bromenshenk, J.J., S.R. Carlson, J.C. Simpson, J.M. Thomas. 1985. Pollution monitoring in puget sound with honey bees. Science 227:632-634.

Brusick, D. 1980. Protocol 13: Bone marrow cytogentic analysis in rats. In: Principles of Genetic Toxicology. Plenum Press. New York, NY.

Buttler, B. 1987. Peromyscus (Rodentia) as environmental monitors: A bibliography. Canadian Union College, Biology Department, College Heights, Alberta, Canada.

Callahan, C., L.K. Russell, and S.A. Peterson. 1985. A comparison of three earthworm bioassay procedures for the assessment of environmental samples containing hazardous wastes. Biol. Fert. Soils. 1:195-200.

Cholakis, J.M., M.J. McKee, L.C.K. Wong, and J.D. Gile. 1981. Acute and subacute toxicity of pesticides in microtine rodents. Pages 143-154. In: D.W. Lamb and E.E. Kenaga, eds. Avian and Mammalian Wildlife Toxicology: Second Conference. ASTM STP 757. American Society for Testing and Materials, Philadelphia, PA.

Edwards, C.A., and J.R. Lofty. 1972. Biology of Earthworms. Chapman and Hall, Ltd., London.

Fava, J.A., W.J. Adams, R.J. Larson, G.W. Dickson, K.L. Dickson, and W.E. Bishop. 1987. Research priorities in environmental risk assessment. Workshop Report. Society of Environmental Toxicology and Chemistry, Rockville, MD.

Gano, K.A., D.W. Carlile, and L.E. Rogers. 1985. A harvester ant bioassay for assessing hazardous chemical waste sites. PNL-5434. Pacific Northwest Laboratory, Richland, WA.

Goats, G., and C.A. Edwards. 1982. Testing the toxicity of industrial chemicals to earthworms. Pages 104-105. In: Rothamsted Exp. Station Report, 1982.

Grant, W.F., and K.D. Zura. 1982. Plants are sensitive *in situ* detectors of atmospheric mutagens. Pages 407-434. In: J.A. Heddle, ed. Mutagenicity: New Horizon in Genetic Toxicology, Academic Press, New York, NY.

Greene, J.C., C.L. Bartels, W.J. Warren-Hicks, B.R. Parkhurst, G.L. Linder, S.A. Peterson, and W.E. Miller. 1988a. Protocols for short-term toxicity screening of hazardous waste sites. U.S. Environmental Protection Agency, Corvallis, OR.

Greene, J.C., W.E. Miller, M. Debacon, M.A. Long, and C.L. Bartels. 1988b. Use of *Selenastrum capricornutum* to assess the toxicity potential of surface and ground water contamination caused by chromium waste. Environ. Toxicol. Chem. 7:35-39.

Horning, W.B., and C.I. Weber. 1985. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. EPA/600/4-85/014. Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH.

Lower, W.R., V.K. Drobney, B.J. Aholt, and R. Politte. 1983. Mutagenicity of the environments in the vicinity of an oil refinery and a petrochemical complex. Terat. Carcinog. Mutagen. 3:65-73.

Lower, W.R., S.S. Sandhu, and M.W. Thomas. 1988. Utility of *in situ* assays for detecting environmental pollutants. In: Proceedings, U.S. EPA Fourth Annual Symposium: Waste Testing and Quality Assurance, Washington, DC.

Ma, T.-H. and M.M. Harris. 1985. *In situ* monitoring of environmental mutagens. Hazard Assess. Chem. 4:77-105.

McBee, K. 1985. Chromosomal aberrations in resident small mammals at a petrochemical waste dump site: A natural model for analysis of environmental mutagenesis. Ph.D. disseration. Texas A&M University, College Station, TX.

McBee, K., J.W. Bickhma, K.W. Brown, and K.C. Donnelly, 1987. Chromosomal aberrations in native small mammals (*Peromyscus leucopus* and *Sigmodon hispidus*) at a petrochemical waste disposal site: I. Standard karyology. Arch. Environ. Contam. Toxicol. 16:681-688.

McCann, J.A., W. Teeters, D.J. Urban, and N. Cook. 1981. A short-term dietary toxicity test on small mammals. Pages 132-142. In: D.W. Lamb and E.E. Kenaga, eds. Avian and Mammalian Wildlife Toxicology: Second Conference. ASTM STP 757, American Society for Testing and Materials, Philadelphia, PA.

Miller, W.E., S.A. Peterson, J.C. Greene, and C.A. Callahan. 1985. Comparative toxicology of laboratory organisms for assessing hazardous waste sites. J. Environ. Qual. 14:569-574.

Morrill, L.G., B.C. Mahilum, and S.H. Mohiuddin. 1982. Organic compounds in soil: Sorption, degradation, and persistence. Ann Arbor Science Publishers, Inc. The Butterworth Group, Ann Arbor, MI.

Peltier, W.H., and C.I. Weber. 1985. Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms. Third Edition. EPA/600/4-85/013. US Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH.

Popham, J.D., and J.M. Webster. 1979. Cadmium toxicity in the free-living nematode Caenorhabditis elegans. Environ. Res. 20:183-191.

Popham, J.D., and J.M. Webster. 1982. Ultrastructural changes in Caenorhabditis elegans (nematoda) caused by toxic levels of mercury and silver. Ecotoxicol. Environ. Saf. 6:183-189.

Redei, G.P., and S.S. Sandhu. 1988. Aneuploidy detection with a short-term hexaploid wheat assay. Mutat. Res., Special Issue on Aneuploidy. In Press.

Rowley, M.H., J.J. Christian, D.K. Basu, M.A. Pawlikowski, and B. Paigen. 1983. Use of small mammals (voles) to assess a hazardous waste site at Love Canal, Niagara Falls, New York. Arch. Environ. Contam. Toxicol. 12:383-397.

Samoiloff, M.R., S. Schulz, Y. Jordan, K. Denich, and E. Arnott. 1980. A rapid simple long-term toxicity assay for aquatic contaminants using the nematode Panagrellus redivivus. Can. J. Fish. Aquat. Sci. 37:1167-1174.

Schafer, Jr., E.W. and W.A. Bowles, Jr. 1985. Acute oral toxicity and repellency of 933 chemicals to house and deer mice. Arch. Environ. Contam. Toxicol. 14:111-129.

Thomas, J.M., and J.E. Cline. 1985. Modification of the Neubauer technique to assess toxicity of hazardous chemicals. In: soils. Environ. Toxicol. Chem. 4:201-207.

Thomas, J.M., J.F. Cline, C.E. Cushing, M.C. McShane, J.E. Rogers, L.E. Rogers, J.C. Simpson, and J.R. Skalski. 1983. Field evaluation of hazardous waste site bioassessment protocols, Volume 1. PNL-4614. Pacific Northwest Laboratory, Richland WA.

Thomas, J.M., J.F. Cline, K.A. Gano, M.C. McShane, J.E. Rogers, L.E. Rogers, J.C. Simpson, and J.R. Skalski. 1984. Field evaluation of hazardous waste site bioassessment protocols, Volume 2. PNL-4614, Vol. 2. Pacific Northwest Laboratory, Richland, WA.

Thomas, J.M., J.R. Skalski, J.F. Cline, M.C. McShane, J.D. Simpson, W.E. Miller, S.A. Peterson, C.A. Callahan, and J.C. Greene. 1986. Characterization of chemical waste site contamination and determination of its extent using bioassays. Environ. Toxicol. Chem. 5:487-501.

Tice, R.R., B.G. Ormiston, R. Boucher, C.A. Luke, and D.E. Paquette. 1987. Environmental biomonitoring with feral roden species. In: Short-term bioassays in the analysis of complex environmental mixtures. V. (Sandhu, S.S., D.M. Demarine, M.J. Mass, M.M. Moore, and J.L. Mumford, eds.) Plenum Press. New York, NY.

Thompson, R.A., G.D. Schroder, and T.H. Connor. 1988. Chromosomal aberrations in the cotton rat, Sigmodon hispidus, exposed to hazardous waste. Envirn. Molec. Mutagen. 11:359-367.

U.S. Environmental Protection Agency. 1985. Toxic Substances Control Act Test Guidelines; Final Rules. 40 CFR parts 796, 797, and 798.

U.S. Environmental Protection Agency. 1982a. Environmental effects test guidelines. EPA 560/6-82/002. U.S. Environmental Protection Agency, Washington, DC.

U.S. Environmental Protection Agency. 1982b. Pesticide assessment guidelines. EPA/ 540/9-8/018 through 028. Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington, DC.

U.S. Environmental Protection Agency. 1982c. Toxic substances test guidelines. EPA/ 6/82/001 through 003. Office of Toxic Substances, U.S. Environmental Protection Agency, Washington, DC.

Walton, B.T. 1980. Differential life-stage susceptibility of Acheta deomesticus to acridine. Environ. Entomol. 9:18-20.

Wilson, M.V., E.R. Ingham, C.D. McIntire, and M.L. Scott. 1987. Report on the selection of several potentially critical terrestrial systems. Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR.

## 6.4 MICROBIAL TOXICITY TESTS -- Gabriel Bitton, Bernard J. Dutka, and Charles W. Hendricks

### 6.4.1 Introduction

Microbes are ubiquitous in the environment and have the capacity to process substrates found in water and soil for their own maintenance and growth but also carry out critical functions necessary for ecosystem stability, some of which are beyond the ability of higher life forms. Because of these unique physiological characteristics, certain microbial species have been utilized in both short-term toxicological testing and to study the effects of pollutants on the cycling of carbon, nitrogen, sulfur, and phosphorus in ecosystems.

Short-term microbial tests are based on inhibition of activities of bacteria, algae, and fungi, and are versatile and cost-effective assessment tools (Hicks and Van Voris 1988; Bitton and Dutka 1986; Dutka and Bitton 1986; Liu and Dutka 1984). Because they are simple, rapid, and relatively inexpensive procedures, they are readily adaptable to miniaturization and automation. Microbial test methods have been developed that assess the toxicity of domestic and industrial effluents, discharges, and waste products. However, with the increasing awareness of the long-term effects of chemicals discharged into aquatic systems and landfill sites, recent research efforts have been directed to the development of short-term bioassay tests to alert regulatory and monitoring agencies, as well as dischargers, of the presence of toxicants in effluents and the aquatic ecosystem (Bulich 1979; Dutka and Kwan 1988).

Ecological effects tests are mainly used to measure the acute toxicity of chemicals to bacteria and other organisms that represent various trophic levels that mediate the cycling of nutrients. These tests aid in the estimation of effects to the stability of ecosystems. These tests can readily be used to assess a wide range of toxicants in waters, soil, sediments, sewage effluents and leachates, either directly or after concentration and/or extraction.

Various microbial toxicity assays have been identified as Class I methods for conducting ecological assessments at hazardous waste sites because these procedures are widely accepted and the methods are of known quality; Class II methods have not been thoroughly investigated under field conditions, but warrant consideration within a site-specific context.

### 6.4.2 Microbial Toxicity Test Methods

### 6.4.2.1 Sample Preparation

6.4.2.1.1 Aqueous Samples. Leachate or surface water samples are usually tested in their natural state or concentrated. Concentration procedures (such as flash evaporation are commonly used, but the procedure may result in the loss of volatile toxicants. Samples may be refrigerated and tested within two to three days of collection, or frozen at -60° C if there is a longer time delay.

6.4.2.1.2 Sediment Samples. Sediments may be collected by Ekman dredge, Ponar grab or other suitable instruments. At each site the collected surface layers (1 to 2 cm) are pooled (usually in a stainless steel bowl), and mixed, and aliquots are dispensed in appropriate containers and stored at melting-ice-temperature until extraction procedures can be initiated.

6.4.2.1.3 Extraction Procedures. Two simple commonly used extraction procedures, water extraction and organic solvent extraction, are performed sequentially on the same sediment sample.

(A) Water Extraction. A portion of sediment (e.g., 100 g) is extracted with very high quality deionized-filtered water. The sample is mixed with water in a 1:1 ratio, shaken vigorously for three to five minutes, then spun at 5000 rpm in a refrigerated centrifuge for 10 minutes. The supernatant is used for toxicity screening tests immediately or frozen until required (Dutka et al. 1988).

(B) Solvent Extraction. The 100 g portion of the above water-extracted sediment is freeze-dried, then weighed on fired aluminum foil (i.e., 550° C overnight). The weighed, freeze-dried sediment is added along with 250 ml dichloromethane (DCM) into a 1-L Erlenmeyer flask, which has been rinsed twice with DCM, and shaken approximately 24 hours on a Burrel wrist action shaker at position 2. After settling overnight, the sediment-solvent mixture is filtered overnight through washed $Na_2SO_4$. One ml of 100% DMSO is added to the filtrate and the mixture is evaporated in a rotary evaporator to 1.0 ml. The sample is transferred into a test tube along with 2 ml DCM rinsings (twice) of the flask. The DCM is evaporated under nitrogen in a water bath to 1.0 ml. This 1.0 ml of 100% DMSO is used in all toxicity screening tests at the 1% level. A solvent blank is prepared for each series of tests containing 250 ml DCM plus 1.0 ml of 100% DMSO evaporated to 1.0 ml DMSO. A method blank is also prepared as control, containing 250 ml DCM plus 1.0 ml DMSO, shaken, filtered and evaporated as per the procedure for the total sample (Dutka and Kwan 1988). DMSO sample preparations may be preserved by freezing at -60° C and may be stored at least four months until analyzed.

Other procedures can be used to concentrate water and extract sediments for toxicant activity tests. The procedures outlined above are provided as one approach.

### 6.4.2.2 ATP Assays

Adenosine triphosphate (ATP), a product of catabolic reactions, is found in all living cells. The fact that ATP is rapidly destroyed after cell death makes it ideal for distinguishing between live and dead cells. The basic assay of ATP consists of measuring the light emission following the reaction of firefly luciferin with ATP in the presence of luciferase and $Mg^{2+}$ (Holm-Hansen 1973).

#### 6.4.2.2.1 Class I ATP Tests

The recommended ATP assay for conducting environmental assessments at hazardous wastes sites is the ATP-TOX system test, developed by Xu and Dutka (1987). Concentrations of ATP in bacterial cells remain relatively constant and stable throughout all phases of growth (D'Eustachio and Johnson 1968); thus, bacterial densities can be estimated by measuring the ATP content of the test system. Growth inhibition usually occurs when rapidly growing bacterial cells are exposed to toxicants. After several life cycles, the toxic effect can be estimated by comparing sample cell growth to a control by measuring the ATP content.

### 6.4.2.3 Enzymatic Activity

Since enzymes are key catalysts for metabolic reactions in cells, their inhibition by environmental toxicants could be the underlying cause of toxicity to the cells. Enzyme inhibition as a basis for toxicity testing has been explored for a wide range of enzymes with special emphasis on the dehydrogenase enzymes (Bitton and Koopman 1986; Christensen et al. 1982). Other enzymes studied include ATPases, esterases, phosphatases, amylase, protease, beta-glucosidase, urease, and luciferase (Obst et al. 1988). Although enzymes are quite sensitive to heavy metals, they generally display little sensitivity to organic toxicants.

One approach to toxicity testing has been to measure the effect of toxicants on the de novo enzyme biosynthesis in microorganisms. The classic example is the inducible enzyme system beta-galactosidase, which is controlled by the cluster of genes known as the lac operon (Jacob and Monod 1961). Toxicity assays based on the inhibition of beta-galactosidase in E. coli have been developed and found to respond well to toxicants (Dutton et al. 1988; Reinhartz et al. 1987). The test based on the inhibition of beta-galactosidase activity is only sensitive to heavy metals, but the one based on enzyme biosynthesis responds to both organic and inorganic toxicants (Dutton et al. 1988).

A modification of this test system has also been used for genotoxicity. This test is based on the induction of the gene sfiA, which is controlled by the general repressor of the SOS system in E. coli. Expression of the sfiA is monitored by a gene fusion with lacZ gene for beta-galactosidase. Comparison of test results with the Ames test showed that most of the mutagenic compounds ( 90% of 83 chemicals of several different classes) were also SOS inducers (Quillardet and Hofnung 1985).

### 6.4.2.3.1 Class I Enzymatic Activity Test.

The Toxi Chromotest and SOS Chromotest are effective for conducting environmental assessments of hazardous waste sites, and consist of colorimetric assays of microbial enzymatic activities after incubating various concentrations of water or sediment and soil extracts with the special test strain E. coli (K-12 PQ37).

These tests, which are available under the trade names of Toxi Chromotest and SOS Chromotest (Orgenics Ltd., Yavne, Israel, and distributed by Colonics Corp., Boulder, CO), provide data on acute toxicity and potential genotoxic effects.

#### 6.4.2.3.2 Class II Enzymatic Activity Test.

A variety of techniques are available to measure changes in dehydrogenase activity as a result of chemical effect on microorganisms. These include measuring color changes of tetrazolium dyes and resazurin, and the direct inhibition of specific dehydrogenase enzymes (Bitton and Koopman 1986). The latter method is well standardized and is available in kit form from Sigma Chemical Co., St. Louis, MO.

The *in vitro* dehydrogenase activity test measures the reduction of NADP to NADPH using glucose-6-phosphate as substrate. NADPH can be measured colorimetrically or with a spectrophotometer. In the colorimetric test, NADPH in the presence of phenazine metasulfate reduces a blue dye to a colorless state. The rate of the disappearance of the blue color is proportional to the dehydrogenase activity. The spectrophotometric test is based on the increased absorbance of NADPH at 340 nm.

### 6.4.2.4 Bioluminescence Assays

Bioluminescence is a branch of the electron transport system, and several investigators have described toxicity assays based on inhibition of this system (Bulich 1984, 1986). The first commercial toxicity test using bioluminescent bacteria was developed at Beckman Instruments, Carlsbad, CA (Bulich 1979, 1982). The test, now marketed by Microbics Corp. (still under the trade name of Microtox), utilizes freeze-dried cultures of the marine bacterium *Photobacterium phosphoreum* and is based on the inhibition of bioluminescence by toxicants. The results of several studies of pure compounds and complex chemical mixtures have revealed that

Microtox is in general agreement with the standard fish and invertebrate bioassays (Curtis et al. 1982; Sanchez et al. 1988).

The presence of 2% sodium chloride in the assay medium can be a problem with Microtox assay. The salt concentration (1 to 7% NaCl) in the assay milieu may readily affect the toxicity of heavy metals such as cadmium or zinc (Hinwood and McCormick 1987). It was proposed that 20.4% sucrose should be added to the assay medium in lieu of 2% NaCl to provide osmotic protection to Photobacterium phosphoreum. Heavy metal toxicity was higher in the presence of sucrose (Hinwood and McCormick 1987). Another concern is that Microtox may not be sensitive to extremely hydrophobic compounds (Hermans et al. 1985).

Notwithstanding these problems, Microtox is a Class I bioluminescence assay for use in conducting environmental assessments of hazardous waste sites. Algal-Tox is recommended as a Class II test. Brief descriptions of these methods are presented in the following subsections.

### 6.4.2.4.1 Class I Bioluminescence Test.

Beckman Instruments, Inc., has developed a test for measuring acute toxicants in water and sediment and soil extracts which utilizes specialized strains of luminescent bacteria (Photobacterium phosphoreum). This test measures the effect of toxic materials (and stimulants) on the metabolism of the culture. Any alteration of cellular metabolism affects the intensity of light output from the organism. When these changes in light output are sensed, the presence and relative concentration of toxicants can be obtained by establishing EC50 levels from plotted data. The EC50 is defined as that concentration of toxicant causing a 50% reduction in light intensity.

#### 6.4.2.4.2 Class II Bioluminescence Test.

The algal ATP toxicant screening test is based on the inhibition of ATP production in cultures of the green alga Selenastrum capricornutum (Blaise et al. 1986). The ATP content of the stressed Selenastrum is measured by the procedure described in Turner (1983). The results are reported as a percentage of relative light output (RLO) of the non-stressed controls (100%).

### 6.4.2.5 Microbial Growth Assays

Algae and photosynthetic bacteria appear to be more susceptible to the action of chemicals than other toxicity test species (e.g., heterotrophs), probably because many of the compounds that have been tested inhibit photosynthesis. Actinomycetes and saprophytic fungi appear to be more resistant to the action of xenobiotics and an increase in their number was detected for many of the compounds tested (Simon-Sylvestre and Fournier 1979). Similar observations have been made for heterotrophic bacteria. In general, compounds such as fungicides have a broad inhibitory effect, causing reduced population densities among all microbial groups. For certain groups of heterotrophic bacteria, this effect can be transient and populations will recover to pretreatment population densities, or above. This increase is usually attributed to the utilization of microbial cells killed by xenobiotic by the surviving organisms.

Microbial populations in the rhizosphere comprise a particularly important soil microbial community. Because of their unique relationship to the plant root zone that they colonize, rhizosphere microbial populations differ from those in soil not directly associated with roots (Gerhardson and Clarholm 1986). Because of their close association with plants, nutrients are available for the mutual benefit of both populations.

Trappe et al. (1984) have reviewed much of the literature on the effects of agricultural chemicals on mycorrhizal fungi and concluded that observable effects are variable and appear to depend on the type of compound as well as on the type of mycorrhizal fungi. The effects of heavy metals on mycorrhizae are also relatively unknown. However, chromium and cadmium have been shown to be inhibitory (Simon-Sylvestre and Fournier 1979; Babich and Stotzky 1985).

It is difficult to quantify accurately microbial populations in situ because the ecological and physical factors that control the growth of microorganisms in water and soil are not well understood. Therefore, a completely accurate environmental assessment of the effects of xenobiotics on microbial populations and communities is not currently possible. Consequently, the quantification of microbial populations in soil and water as a measure of the effect of xenobiotics on microorganisms is often disregarded (Greaves 1982). Changes in microbial populations, if detectable, can, however, serve as a guide to the interpretation of metabolic data, such as respiration or nitrogen transformations (Grossbard 1976). In addition, results obtained from changes in species composition can aid in the interpretation of data obtained in the environmental assessment.

As a result of these observations, direct microbial growth measurements are not definitive although excellent Class I type methods are available (APHA 1985; ASTM 1987; US EPA 1978). Two microbial assays are discussed below to augment Class I ATP, enzyme activity, and bioluminescence assays discussed in the preceding sections of this chapter.

### 6.4.2.5.1 Microbial Growth Tests.

A. Population Density Measurements. The quantitative estimation of microbial populations provides a general indication of ecosystem stability. While numbers of particular species may vary, a significant reduction or increase in numbers is useful in the interpretation of other information about the site. Particularly important organisms include the rhizosphere bacteria, mycorrhizal fungi and free-living organisms in soil and water at the site. For each group of organisms, a specific growth medium must be used, but standard techniques are available for both water (APHA 1985) and soil (Black 1965).

The traditional approach to toxicity testing is to measure the effect of toxicants on growth inhibition of pure bacterial cultures or mixtures of microorganisms originating from various sources (Alsop et al. 1980; Trevors 1986). The turbidity of the bacterial suspensions is read initially and after 16-hour incubation at room temperature. In the Netherlands, a standard toxicity test is based on growth inhibition of Pseudomonas fluorescens ATTC 13525 (Trevors 1986). More recently, a miniaturized six-hour test based on the growth inhibition of Aeromonas punctata was found to be more sensitive than other bacterial tests evaluated (Slabbert 1988).

B. Spirillium volutans. This test is based on loss of coordination and subsequent loss of bacterial motility in the presence of toxicants (Bowdre and Krieg 1974). It has been extensively used to measure environmental toxicants as well as the toxicity of heavy metal mixtures (Dutka and Kwan 1988) and has been found to be in good agreement with the Daphnia bioassay (Sanchez et al. 1988).

### 6.4.3 "Ecological Effect" Tests

Nutrient cycling is one of the most ecologically significant and potentially most sensitive processes within terrestrial ecosystems. Soil processes involving nutrients, especially those of carbon, nitrogen, phosphorus, and sulfur are important to the well-being and health of ecosystems and contribute to soil stability, soil fertility, and plant productivity. The movement of nutrients in an ecosystem includes cycling within the below-ground and above-ground portions and also between the two components. Such processes are performed by an array of microorganisms including free-living and symbiotic bacteria and fungi, algae, various protozoans, and higher plants and animals.

Because the majority of biochemical transformations in soil result from microbial activity (Alexander 1977), there is concern that waste materials that can affect microbial life may also alter cycling of nutrients in the environment and ultimately affect soil fertility and plant productivity. For example, processes such as nitrification and sulfur oxidation are mediated exclusively by specific groups of microorganisms, and the rates at which their metabolic processes occur is indicative of their activity. The major limitations of assays based on these processes are that (1) little information is available about the specific organism or group of organisms that may be affected by the toxicant, and (2) other tests must be performed if that information is desired (e.g., direct plate count). Nevertheless, these techniques are especially useful in programs designed to assess toxicity (Barkay et al. 1986; Van Voris et al. 1985).

Four nutrient cycling processes that are valuable in the environmental assessment of hazardous waste sites are carbon, nitrogen, sulfur, and phosphorus transformations.

Carbon transformations - The relationship between specific chemicals and their effect on respiration is unclear from the literature; but in general, low concentrations of recalcitrant compounds (such as chlorinated aromatic hydrocarbons) exert little effect on microbial respiration. At higher concentrations, however, chlorinated aromatics are toxic to microorganisms (Boyd and Shelton 1984), and result in respiration inhibition. Less persistent organic compounds, such as the carbamate and phenylurea pesticides, appear to suppress respiration, but the nonselective fungicides appear to do so to the greatest extent (Parr 1974). At low concentrations, other organic xenobiotic compounds have been shown to stimulate oxygen consumption (Grossbard 1976).

Because the respiratory response to toxicants may be either inhibitory or stimulatory, the technique should be used in conjunction with other procedures. The stimulatory effect has been observed even after an initial inhibitory effect and could result from a waste that is biodegradable (Bitton and Dutka 1986), or from the uncoupling of oxidative phosphorylation from the electron transport chain (Bartha et al. 1967), or from the degradation of those organisms that may have been originally sensitive to the waste chemicals (Jenkinson and Powlson 1976).

Respiration is a convenient parameter to consider as a basis for toxicity testing using pure cultures of aerobic bacteria or mixtures of indigenous microorganisms. Several approaches are available for measuring respiration rates, including manometric techniques, titrimetric method, electrolytic respirometers, oxygen electrodes, and immobilized microorganisms (King and Dutka 1986). Toxicity tests based on inhibition of microbial respiration have long been favored for monitoring sewage treatment plants and polluted surface waters. However, these tests do not appear to be the most sensitive for measuring the impact of toxicants on aquatic and soil environments.

Soil respiration does provide an overall indication of the effects of toxicants on soil microbial activities. However, it is also important to determine their effects on the utilization of specific carbon compounds. The initial decomposition of cellulose is generally attributed to soil fungi, and fungicidal compounds appear to have the greatest impact on cellulose degradation (Grossbard 1976). Nonfungicidal compounds, such as herbicides and heavy metals, have also been shown to inhibit cellulose degradation (Wainwright 1978; Martin et al. 1982).

Nitrogen transformations - The transformation of organic nitrogen to inorganic forms is an important microbial function contributing to the fertility of soil and is a microbial process that has become a significant indicator in assessing the effects of toxicants. The major nitrogen transformations mediated by soil microorganisms include ammonification, nitrification, denitrification, and nitrogen fixation.

Nitrobacter has been proposed as a bioassay organism for measuring the toxicity of industrial effluents (Williamson and Johnson 1981) and pesticide impact on soils (Mathes and Schulz-Berendt 1988). While nitrification appears to be the most sensitive part of the nitrogen cycle to the action of toxicants, chlorinated hydrocarbons appear to have minimal effect when applied at low rates. However, chronic effects may result from repeated application of these pesticides. Studies by Carlisle and Trevors (1986) and Rhodes and Hendricks (1988) have shown that nitrification is sensitive to some herbicides, but more information is needed concerning the chronic versus acute effects of toxicants on microorganisms in soils. Degradation products of chlorinated compounds may also influence nitrification

(Corke and Thompson 1970). In general, nitrification is inhibited by the action of heavy metals (Giashuddin and Cornfield 1979; Rother et al. 1982; Chang and Broadbent 1982; Bewley and Stotzky 1983). The comparative toxicity of metals to nitrification follows the sequence, Hg > Cr > Cd > Ni > Cu > Zn > Pb (Liang and Tabatabai 1978).

Sulfur and phosphorus transformations - Sulfur enters soil primarily in the form of plant residues, animal wastes, chemical fertilizers, and rainwater, and a large part of the sulfur in the soil profile is present in organic matter. Sulfate is the principal plant-available source of sulfur. The oxidation of sulfur to sulfate and the reduction of sulfate are particularly important (Alexander 1977; Granat et al. 1976).

Certain pesticides have been shown to decrease sulfur oxidation when added to soils. Tu and Miles (1976) reported that 2000 ppm Aldrin and Eldrin decreased the rate of sulfur oxidation for 2 months, whereas Audus (1970) reported no effect at this concentration. Herbicides such as Paraquat and 2,4-D have been shown to decrease the oxidation of sulfur, although it is not known if the decrease was the result of a direct action on the principal organisms responsible for oxidation or an indirect effect caused by the loss of plant exudates after the death of the plant (Tu and Bollen 1968).

Phosphorus exists in soils as inorganic forms and as organic forms that undergo mineralization (Alexander 1977). Wainwright and Snowden (1977) showed that fungicides increased slightly the level of CaCl2-extractable phosphorus in soils, resulting in increased solubilization of added insoluble phosphates. These increases were associated with an increase in the population of phosphorus-solubilizing bacteria after soil treatment. The application of insecticides and herbicides has been shown to have little effect on either phosphorus mineralization from organic matter or solubilization from inorganic forms (Smith and Weeraratna 1974; Tyunyayeva et al. 1974), but heavy metals appear to inhibit microbially mediated cycling of inorganic phosphorus (Juma and Tabatabai 1977; Capone et al. 1983).

At present no Class I ecological effects methods are available, but two Class II assays are discussed below to augment the core group of recommended microbial assays.

### 6.4.3.1 Class II Ecological Effect Tests

6.4.3.1.1 **Nitrification Inhibition.** The biological oxidation of ammonia to nitrate in soil is facilitated by two groups of chemolithotrophic bacteria: ammonium oxidizers and nitrite oxidizers. Inhibition of either of these groups may significantly alter the dynamics of the soil nitrogen pool. These organisms grow slowly and are difficult to maintain in pure culture. Consequently, most studies utilize nitrifying bacteria naturally present in soil and focus on the impact of toxicants on nitrification rates. Currently, three techniques are used to examine effects of chemicals on nitrification. These are the continuous flow method (Rhodes and Hendricks 1989), the perfusion column (Lees and Quastel 1946), and the static batch culture (Black 1965).

The assays are performed by adding various concentrations of an extract from a contaminated soil or dilutions of a water sample to a nitrifying soil culture. After incubation, static soil cultures are extracted and filtered. Extraction is not necessary for the perfusion and continuous-flow cultures, and the eluates can be analyzed directly without further preparation.

Ammonia, nitrate, and nitrite are measured by standard techniques using automated analysis (U.S. EPA 1979). With these procedures, detection levels for nitrite and nitrate are 0.005 mg/L and 0.01 mg/L for ammonia. When necessary, dilution of soil extracts can be prepared with deionized water.

6.4.3.1.2 Mineralization of Organic Sulfur. The organic forms of sulfur are found extensively in the terrestrial environment, particularly in algae and green plants. Plants are able to degrade sulfolipid primarily to 6-sulfo-6-deoxyglucose. This compound serves as a primary substrate for sulfur-metabolizing soil microflora. The mineralization of organic sulfur compounds can be an effective means for evaluating the response of microorganisms to toxic chemicals in the environment. While this assay is highly sensitive, it does require the use of scintillation counting equipment found in well equipped laboratories.

This procedure (Strickland and Fitzgerald 1983) utilizes the $^{35}S0_4^{2-}$ isotope of 6-sulfo-6-deoxyglucose (Sulfoquinovose). This substrate is incubated with soil for various time periods and extracted to recover mineralized organic and inorganic fractions. These fractions are measured for total radioactive sulfur, from which the rate of mineralization is determined.

To measure the effects of toxicants on the rate of sulfur mineralization, various dilutions of contaminated water or soil extracts are added to an actively growing culture undergoing sulfur mineralization.

### 6.4.4 Case Study: Battery Approach to Toxicity Testing

Some investigators have suggested that a core group of toxicity tests should be used to assess the toxicity of environmental samples (Calleja et al. 1986; Qureshi et al. 1982). An integrated approach to ecotoxicity testing has been followed by researchers from Environment Canada (Blaise et al. 1985; Blaise et al. 1988).

Plotkin and Ram (1984) demonstrated the usefulness of the battery approach for measuring the toxicity of landfill leachates. They recommended a series of toxicity tests with organisms (bioluminescent bacteria, algae, daphnid, and fish) belonging to different trophic levels. A battery of indicator tests was also evaluated at several sites, including landfill sites (Burton and Stemmer 1988). The tests included several enzymatic assays (alkaline phosphatase, protease, amylase, arylsulfatase, dehydrogenase, beta-galactosidase, beta-glucosidase), heterotrophic $^{14}C$ uptake, zooplankton, amphipods, and fish. This approach was recommended for routine ecotoxicity testing.

A battery concept was also adopted for testing the toxicity of sediment extracts (Dutka and Kwan 1988; Giesy et al. 1988). Dutka and Kwan (1988) studied the toxicity of sediments from Lake Ontario, Port Hope Harbour, Canada. Sediments were extracted with very high quality deionized-filtered water or with a solvent (extraction with dichloromethane followed by evaporation and resuspension in dimethylsulfoxide). The toxicity of the sediment extracts was tested using five toxicity assays: Microtox, Spirillium volutans, algal inhibition, ATP-Tox, and Daphnia magna acute mortality test. The toxicity of the sediment water extract was detected only through the Daphnia magna bioassay. However, all the microbial tests showed toxicity in the solvent extracts. This points out the importance of the extraction liquid for sediments and probably soils in toxicity tests. The selection of specific tests to be used in the battery of toxicity screening assays is also critical. For example, a Canada-wide study of water and sediment samples has revealed the importance of test battery makeup, sample type, and extraction procedure (Dutka 1988). For water samples and water extracts of sediments, the optimum tests were Daphnia magna and algal inhibition assays. However, for solvent extracts of sediments, the preferred battery was composed of Microtox and algal inhibition tests.

These studies showed that Microtox bioluminescent bacteria readily respond to hydrophobic compounds from the sediments extracted with dichloromethane.

With careful selection of toxicity screening tests, the battery testing approach will undoubtedly be refined in the near future as our knowledge on the individual toxicity tests expands. It will provide a rapid and low cost means of assessing chemical toxicity in the environment.

### 6.4.5 References

Alsop, G.M., G.T. Waggy, and R.A. Conway. 1980. Bacterial growth inhibition test. J.W.P.C.F. 52:2452.

Alexander, M. 1977. Introduction to Soil Microbiology. 2nd ed. Wiley, New York, NY.

American Public Health Association (APHA). 1985. Standard Methods for the Examination of Water and Wastewater. 16th ed. American Public Health Association, Washington, DC.

ASTM. 1987. Annual Book of ASTM Standards American Society for Testing and Materials. Philadelphia, PA.

Audus, L.J. 1970. The action of herbicides and pesticides on the soil microflora. Meded. Fac. Landbouwwet. Rijksuniv. Gent. 35:465-492.

Babich, H. and G. Stotzky. 1985. Heavy metal toxicity tomicrobe-mediated ecological processes: A review and potential application to regulatory policies. Environ. Res. 14:409-415.

Barkay, T., D.F. Shearer, and B.H. Olson. 1986. Toxicity testing in soil using microorganisms. Pages 133-155. In: B. J. Dutka and G. Bitton, eds. Toxicity Testing Using Microorganisms, Vol. 2. CRC Press, Boca Raton, FL.

Bartha, R., L.D. Lanzillota, and D. Pramer. 1967. Stability and effects of some pesticides in soil. Appl. Microbiol. 15:67-75.

Bewley, R.J.F. and G. Stotzky. 1983. Effects of cadmium and zinc on microbial activity in soil: Influence of clay minerals. Part II: Metals added simultaneously. Sci. Total Environ. 31:57-69.

Bitton, G., and B.J. Dutka, eds. 1986. Toxicity Testing Using Microorganisms, Vol. 1. CRC Press, Boca Raton, FL.

Bitton, G., and B. Koopman. 1986. Biochemical tests for toxicity screening. Pages 27-55. In: G. Bitton and B.J. Dutka, eds. Toxicity Testing Using Microorganisms. CRC Press, Boca Raton, FL.

Black, C.A., ed. 1965. Methods of Soil Analysis, Vol. 2, Chemical and Microbiological Properties. Am. Soc. Agronomy, Madison,WI.

Blaise, C., N. Bermingham, and R. Van Coillie. 1985. The integrated ecotoxicological approach to assessment of ecotoxicity. Water Qual. Bull. 10:3-10.

Blaise, C., R. Legault, N. Bermingham, R. van Coillie, and P. Vasseur. 1986. A simple microplate algal assay technique for aquatic toxicity assessment. Tox. Assessment. 1:261-281.

Blaise, C., G. Sergy, P. Wells, N. Bermingham, and R. Van Coillie. 1988. Biological testing -- development, application, and trends in Canadian environmental protection laboratories. Toxicity Assessment: An International Journal, 3:(in press).

Bowdre, J.A., and N.R. Krieg. 1974. Water Quality Monitoring: Bacteria as Indicators. Virginia Water Resources Research Center, Bull. No. 69, Virginia Polytechnic Institute and State University, Blacksburg, VA.

Boyd, S.A. and D.R. Shelton. 1984. Anaerobic biodegradation of chlorophenols in fresh and acclimated sludge. Appl. Environ. Microbiol. 47:272-277.

Bulich, A.A. 1979. Use of luminescent bacteria for determining toxicity in aquatic environments. In: Markings, L.L., and R.A. Kimerle, eds. Aquatic Toxicology. American Society for Testing and Materials, Philadelphia, PA.

Bulich, A.A. 1982. A practical and reliable method for monitoring the toxicity of aquatic samples. Process Biochem. 17:45-47.

Bulich, A.A. 1984. Microtox: A bacterial toxicity test with several environmental applications. Pages 55-64. In: D. Liu and B. J. Dutka, eds. Toxicity Screening Procedures Using Bacterial Systems. Marcel Dekker, New York, NY.

Bulich, A.A. 1986. Bioluminescent assays. Pages 57-74. In: G. Bitton and B. J. Dutka, eds. Toxicity Testing Using Microorganisms, Vol. 1. CRC Press, Boca Raton, FL.

Burton, G.A., Jr., and B.L. Stemmer. 1988. Evaluation of surrogate tests in toxicant impact assessments. Tox. Assess. 3:255-269.

Calleja, A., J.M. Baldasano, and A. Mulet. 1986. Toxicity analysis of leachates from hazardous wastes via Microtox and Daphnia magna. Toxicity Assess. 1:73-83.

Capone, D.G., D.D. Reese, and D.P. Kiene. 1983. Effects of metals on methanogensis, sulfate reduction, carbon dioxide evolution, and microbial biomass in an anoxic salt marsh sediment. Appl. Environ. Microbiol. 45:1586-1591.

Carlisle, S.M., and J.T. Trevors. 1986. Effects of the herbicide glyphosate on nitrification, denitrification, and acetyl reduction in soil. Water Air Soil Polut. 29:189-203.

Chang, F.H. and F.E. Broadbent. 1982. Influence of trace metals on some soil nitrogen transformations. J. Environ. Qual. 11:1-4.

Christensen, G.M., D. Olson, and B. Reidel. 1982. Chemical effects on the activity of eight enzymes: A review and a discussion relevant to environmental monitoring. Environ. Res. 29:247-255.

Corke, C.T. and F.R. Thompson. 1970. Effects of some phenylamide herbicides and their degradation products in soil nitrification. Can. J. Microbiol. 16:567-571.

Curtis, C., A. Lima, S.J. Lorano, and G.D. Veith. 1982. Evaluation of a bacterial bioluminescence bioassay as a method for predicting acute toxicity of organic chemicals to fish. Pages 170-178. In: J.G. Pearson, R.B. Foster and W.E. Bishop, eds. Aquatic Toxicity and Hazard Assessment, STP 766, American Society for Testing and Materials., Philadelphia, PA.

D'Eustachio, A.J. and D.R. Johnson. 1968. Instrumental approach to rapid microbiology. Internal Pub., E.I. Dupont Nemours and Co., Wilmington, DE.

Dutka, B.J. 1988. A proposed ranking scheme and battery of tests for evaluating hazards in Canadian waters and sediments. National Water Research Institute, Environment Canada, Contribution 88-80, Burlington, Ontario, Canada.

Dutka, B.J., and G. Bitton, eds. 1986. Toxicity Testing Using Microorganisms, Vol. 2. CRC Press, Boca Raton, FL.

Dutka, B.J., and K.K. Kwan. 1988. Battery of screening tests approach applied to sediment extracts. Toxicity Assess. 3:303-314.

Dutka, B.J., K. Jones, K.K. Kwan, H. Bailey and R. McInnis. 1988. Use of microbial and toxicant screening tests for priority site selection of degraded areas in water bodies. Water Res. 22:503-510.

Dutton, R.J., G. Bitton, and B. Koopman. 1988. Enzyme biosynthesis versus enzyme activity as a basis for microbial toxicity testing. Toxicity Assess. 3:245-253.

Gerhardson, B. and M. Clarholm. 1986. Microbial communities and plant roots. Pages19-34. In: V. Jensen, A. Kjoller, and L.H. Sorensen eds.. Microbial Communities in Soil. Elsevier, NY.

Giashuddin, M. and A.H. Cornfield. 1979. Effects of adding nickel (as oxide) to soil on nitrogen and carbon mineralization at different pH levels. Environ. Pollut. 19:67-70.

Giesy, J.P., R.L. Graney, J.L. Newsted, C.J. Rosiu, A. Benda, R.J. Kreis, Jr., and F.J. Horvath. 1988. Comparison of three sediment bioassay methods using detroit river sediments. Environ. Toxicol. Chem. 7:483-498.

Granat, L., R.O. Hallberg, and H. Rodhe. 1976. The global sulfur cycle. In: B.H. Svensson and R. Soderlund, eds. Nitrogen, Phosphorus, and Sulfur -- Global Cycles, SCOPE Report 7. Ecol. Bull. (Stockholm). 22:23-73.

Greaves, M.P. 1982. Effect of pesticides on soil microorganisms. Pages 613-630. In: R.G. Burns and J.H. Slater, eds. Experimental Microbial Ecology. Blackwell Scientific Publications, London.

Grossbard, E. 1976. Effects on the soil microflora. Pages 99-147. In: L.J. Audus, ed. Herbicides: Physiology, Biochemistry, and Ecology. Academic Press, New York, NY.

Hermans, J., F. Busser, P. Leevwangh and A. Musch. 1985. Quantitative structure-activity relationships and mixture toxicity of organic chemicals in Photobacterium phosphoreum: The Mirotox test. Ecotoxicol. Environ. Safety. 9:17-25.

Hicks, R.J. and P. Van Voris. 1988. Review and Evaluation of the Effects of Xenobiotic Chemicals on Microorganisms in Soil. Report 6186. Pacific Northwest Laboratory, U.S. Department of Energy, Battelle Memorial Institute, Richland, WA.

Hinwood, A.L., and M.J. McCormick. 1987. The effect of ionic solutes on $EC_{50}$ values measured using the Microtox test. Toxicity Assess. 2:449-461.

Holme-Hansen, O. 1973. Determination of total microbial biomass by measurements of 90 adenosine triphosphate. In: Stevenson L.H. and R.R. Lowell, eds. Estuarine Microbial Ecology. University of South Carolina Press, Columbia, SC.

Jacob, F. and J. Monod. 1961. Genetic regulatory mechanisms in the synthesis of proteins. J. Mol. Biol. 3:318-356.

Jenkinson, D.S., and D.S. Powlson. 1976. The effects of biocidal treatments on metabolism in soil. Part V: A method for measuring soil biomass. Soil Biol. Biochem. 8:209-213.

Juma, N.G., and M.A. Tabatabi. 1977. Effects of trace elements on phosphatase activity in soils. Soil Sci. Soc. Amer. J. 41:343-346.

King, E.F., and B.J. Dutka. 1986. Respirometric techniques. Pages 75-113. In: G. Bitton and B. J. Dutka, eds. Toxicity Testing Using Microorganisms, Vol. 1. CRC Press, Boca Raton, FL.

Lees, H. and J.H. Quastel. 1946. Biochemistry of nitrification in soil. I. Kinetics of, and the effect of poisons on, soil nitrification, as studied by a soil perfusion technique. Biochem. J. 40:803-814.

Liang, C.N., and M.A. Tabatabai. 1978. Effects of trace elements on nitrification in soils. J. Environ. Qual. 7:291-293.

Liu, D. and B.J. Dutka, Eds. 1984. Toxicity Screening Procedures Using Bacterial Systems. Marcel Dekker, New York, N.Y.

Martin, M.H., E.M. Duncan, and P.J. Coughtrey. 1982. The distribution of heavy metals in a contaminated woodland ecosystems. Environ. Pollut. Sci. B 3:147-157.

Mathes, K. and V.M. Schulz-Berendt. 1988. Ecological risk assessment of chemicals by measurements of nitrification combined with a computer simulation model of the N-cycle. Toxicity Assess. 3:271-286.

Obst, U., A. Holzapfel-Pschorn, and M. Wiegand-Rosinus. 1988. Application of enzyme assays for toxicological water testing. Toxicity Assess. 3:81-91.

Parr, J.F. 1974. Effects of pesticides on microorganisms in soil and water. Pages 315-340. In: W.D. Guenzi, ed. Pesticides in Soil and Water. Soil Sci. Soc. Amer., Madison, WI.

Plotkin, S., and N.M. Ram. 1984. Multiple bioassays to assess the toxicity of a sanitary landfill leachate. Arch. Environ. Contam. Toxicol. 13:197-206.

Quillardet, P., and M. Hofnung. 1985. The SOS chemotest, a colorimetric bacterial assay for genotoxins: Procedures. Mutation Res. 147:65-78.

Qureshi, A., K.W. Flood, S.R. Thompson, S.M. Janhurst, C.S. Inniss, and D.A. Rokosh. 1982. Comparison of a luminescent bacterial with other bioassays for determining toxicity of pure compounds and effluents. p. 179-195. In: J.G. Pearson, R.B. Foster, and W.E. Bishop, eds. Aquatic Toxicology and Hazard Assessment, 5th. Conf. STP 766. American Society for Testing and Materials (ASTM), Philadelphia, PA.

Reinhartz, A., I. Lampert, M. Herzberg, and F. Fish. 1987. A new, short-term, sensitive bacterial assay kit for the detection of toxicants. Toxicity Assess. 2:193-206.

Rhodes,A.N. and C.W. Hendricks. 1989. A continuous-flow method for measuring effects of chemicals on soil nitrification. Toxicity Assess. In Press.

Rother, J.A., J.W. Millbank, and I. Thornton. 1982. Seasonal fluctuations in nitrogen fixation (acetylene reduction) by free-living bacteria in soils contaminated with cadmium, lead, and zinc. J. Soil. Sci. 33:101-113.

Sanchez, P.S., M.I.Z. Sato, C.M.R. B. Paschoal, M.N. Alves, E.V. Furlan, and M.T. Martins. 1988. Toxicity assessment of industrial effluents from Sao Paulo state, Brazil, using short-term microbial assays. Toxicity Assess. 3:55-80.

Slabbert, J.L. 1988. Microbial toxicity assays used for water quality evaluation in South Africa. Toxicity Assess. 3:101-115.

Simon-Sylvestre, G., and J.C. Fournier. 1979. Effects of pesticides on the soil microflora. Adv. Agron. 31:1-92.

Smith, M.S., and C.W. Weeraratna. 1974. The influence of some biologically active compounds on microbial activity and on availability of plant nutrients in soils. Pest. Sci. 5:721- 729.

Strickland,T.C., and J.W. Fitzgerald. 1983. Mineralization of sulfur in sulfoquinovose by forest soils. Soil Biol. Biochem. 15:347-349.

Trappe, J.M., R. Molina, and M. Castellano. 1984. Reactions of mycorrhizal fungi and mycorrhiza formation to pesticides. Ann. Rev. Phytopathol. 22:331-359.

Trevors, J.T. 1986. Bacterial growth and activity as indicators of toxicity. Pages 9-25. In: G. Bitton and B.J. Dutka, eds. Toxicity Testing Using Microorganisms, Vol. 1. CRC Press, Boca Raton, FL.

Tu, C.M., and W.B. Bollen. 1968. Effect of paraquat on microbial activities in soils. Weed Res. 8:28-31.

Tu, C.M., and J.R.W. Miles. 1976. Interactions between insecticides and soil microbes. Res. Rev. 64:5-65.

Turner Design. 1983. Luminescence Rev. Bull. #204. Turner Design, Mountain View, CA.

Tyunyayeva, G.N., A.K. Minenko, and L.A. Ponkov. 1974. Effect of trifluralin on the biological properties of soil. Soviet Soil Sci. 6:320-324.

U.S. Environmental Protection Agency. 1978. Microbiological methods for monitoring the environment. EPA 600/8-78-017. Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH.

U.S. Environmental Protection Agency. 1979. Methods for the chemical analysis of water and wastes. EPA-600/4-79-020. Environmental and Sampling Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH.

Van Voris, P., D.A. Tolle, M.F. Arthur, and J. Chesson. 1985. Terrestrial microcosms: Applications, validation, and cost-benefit analysis. Pages 117-143. In: J. Cairns, Jr., ed. Multispecies Toxicity Testing. Pergamon Press, New York, NY.

Wainwright, M. 1978. A review of the effects of pesticides on microbial activity in soils. J. Soil Sci. 287-298.

Wainwright, M., and F.J. Snowden. 1977. Influence of fungicide treatment on $CaCl_2$-extractable phosphorus and phosphate solubilizing microorganisms. Plant Soil. 48:334-345.

Williamson, K.J., and D.G. Johnson. 1981. A bacterial bioassay for assessment of wastewater quality. Water Res. 15:383.

Xu, H. and B.J. Dutka. 1987. ATP-TOX systyem: A new rapid sensitive bacterial toxicity screening system based on the determination of ATP. Toxicity Assess. 2:149-166.

# CHAPTER 7

# BIOMARKERS

By

Richard T. DiGiulio, School of Forestry and Environmental Studies,
Duke University, Durham, NC.

## 7.1 INTRODUCTION

The concept of "biomarkers" has recently received considerable attention among ecotoxicologists as a potentially powerful approach for assessing environmental degradation, particularly due to anthropogenic contaminants. The underlying concept is that selected endpoints measured in individual organisms, typically comprised of biochemical or physiological responses, can provide sensitive indices of exposure or, more importantly, sublethal stress. In this chapter, selected biomarkers for exposure, including bioaccumulation, and sublethal stress are described. The biomarkers described have been selected based on their present availability for routine monitoring and their applicability to hazardous waste site evaluations. The former criterion greatly limits the number of biomarkers warranting discussion at this time. However, it must be kept in mind that this approach comprises an extremely active area of research and, consequently, the list of available biomarkers will be considerably expanded in the next several years.

When monitoring for adverse environmental effects due to toxicants emanating from hazardous waste sites, it should be noted that biomarkers cannot be used currently to ascertain effects at the biological levels of organization of greatest ecological concern, (i.e., population, community, and ecosystem levels). However, carefully selected biomarkers may serve as very sensitive monitoring tools for detecting exposure and sublethal stress and provide examples, an early warning system for adverse ecological effects and an approach for delimiting zones of impact. Furthermore, there is concern over the sensitivity of the endpoints available for determining population-ecosystem level effects. Endpoints such as density, diversity, or nutrient cycling rates typically display such high natural variability that contaminant-mediated impacts may have to be severe for them to show change. The often greater sensitivity of biomarkers may be due to lower inherent variability, as well as their typically closer relationships to mechanisms of action. Additionally, the biomarker approach has considerable potential for assisting with human health hazard assessments, where individual organism responses are of great concern. In this context, animals inhabiting waste sites, or exposed to waste site media, can serve as sentinels for health effects in humans.

Criteria for useful biomarkers include sensitivity, reliability, feasibility, and applicability to hazardous waste site environments. The issue of sensitivity is particularly important because a key rationale using biomarkers, particularly for sublethal stress, is the potential they have for detecting effects at earlier stages than most other approaches. In this regard, biomarkers that are closely related to biochemical mechanisms of action are likely to be more sensitive than more general indices of stress. However, "nonspecific" indices of stress may still be useful, particularly when mechanisms are unknown or do not yield usable markers. In the context of hazardous waste sites, biomarkers that are relatively compound- or mode of action-specific, as well as more nonspecific indices, are both likely to be useful. Given the very complex nature of some hazardous waste site contaminant mixtures, nonspecific indices may prove to be more useful than they are often considered.

The biomarker approach is readily incorporated into both laboratory toxicity testing and field studies. Many laboratory studies can easily be designed to allow for the examination of selected biomarkers. Any required modification in the design of laboratory studies will depend on the biomarkers selected for examination. Important considerations here include tissue requirements (for example, some markers may require more tissue than normally provided in some routine toxicity tests) and duration of exposure (some biomarkers require longer exposure times than provided by acute toxicity tests). Biomarker measurements can also be made in conjunction with field studies that provide for sampling of organisms. Such studies may involve either sampling of free-living organisms or in situ exposures of "controlled" organisms. Important general considerations here include the availability of suitable reference sites, the frequent necessity of destructive sampling, and the considerable care generally required in sample handling.

Biomarkers can play an important role in integrating results from laboratory and field studies. For example, dose-response relationships can be elucidated in laboratory studies for selected biomarkers (such as bioaccumulation, enzyme activities, etc.). Then, the subsequent measurement of the biomarkers in field studies will provide important information regarding "effective" (i.e., causing effects) environmental concentrations of contaminants on the site(s) of interest. Conversely, the measurement of an array of biomarkers in conjunction with field studies can direct the choice of which biomarkers are examined in detailed laboratory studies.

Many biomarkers that are considered to be feasible and applicable to hazardous waste sites are described in the following sections of this chapter. A few biomarkers that are included may be insufficiently developed for routine monitoring, but may be useful in particular situations. The biomarkers that are discussed have been chosen from other potential techniques based on the criteria described previously; however, a degree of subjectivity was also operative. Individuals using this approach are encouraged to watch both for the full development of additional biomarkers and for other, existing biomarkers of utility for a particular problem at a site under investigation. The biomarkers described in this chapter have been divided into the following two major categories: (1) markers for exposure, and (2) markers for sublethal stress. However, overlap between these categories occurs and is noted.

## 7.2 BIOMARKERS FOR EXPOSURE

The most direct way to assess exposure to contaminants is to measure tissue residues, a key component of bioaccumulation. When feasible, this approach is recommended. However, when measuring tissue residues is not feasible such as with compounds that do not readily bioaccumulate (due to rapid metabolism, for example) or with complex mixtures that require time and cost intensive analyses that may not identify all toxic chemicals, indirect measures of exposure may be required or preferred. An additional attraction of indirect measures, which are typically biochemical endpoints, is that they indicate a biological response to the exposure that is often of toxicological significance; tissue residues alone convey no such information. Such biochemical endpoints blur the distinction between indices of exposure and response, and are more integral to the concept of "biomarkers" than tissue residues.

### 7.2.1 Direct Indices of Exposure

The following discussion of biomarkers for exposure is divided into a section dealing with direct measures (i.e., bioaccumulation) and a section dealing with indirect measures (i.e., biochemical responses). These categories are further subdivided into separate subsections for the two classes of compounds of greatest concern at waste sites -- trace metals and organics. Class I and Class II test methods are identified, where appropriate.

In each subsection, techniques for measuring biomarkers are discussed, along with considerations regarding species and tissue selection, data analysis and interpretation, and quality assurance and quality control. At the conclusion of each biomarker-toxicant section, example case studies are provided.

### 7.2.1.1 Class I Methods: Trace Metals

7.2.1.1.1 **Species Selection.** In monitoring bioaccumulation of trace metals (and perhaps many organics as well), the appropriate species and tissues to analyze are often more difficult questions to resolve than the analytical technique. Decisions here, particularly regarding species selection, will be largely influenced by the ecology of the site and information about contaminating metals. It is important to note, however, that trace metals generally do not display biomagnification, and physical positioning in the environment appears more important than trophic position in determining exposure. Typically, soil- or sediment-inhabiting organisms display the greatest tissue concentrations of contaminating metals (for example see Mathis et al. 1979). Therefore, for biomonitoring of trace metal contamination, soil-associated terrestrial organisms or tissues (such as earthworms, small burrowing mammals, and roots of plants) and benthic aquatic organisms (including bivalves, bullheads, and rooted macrophytes) are often chosen. Mercury, due to its propensity to undergo methylation and thereby become relatively lipophilic, is an exception and has demonstrated biomagnification (Jernelov 1972). For this metal, therefore, species occupying higher trophic positions are generally preferred. It is important to keep in mind the distinction between bioaccumulation and effects during species selection. Those organisms demonstrating the greatest tissue residues are by no means necessarily those most likely to be affected. Species sensitivity, when known, may also play a key role in selecting organisms for residue analysis. (In addition, see Section 8.5.2.2.1.)

7.2.1.1.2 __Tissue Selection.__ Typically in animals the liver is the tissue of choice, often followed by the kidneys, for assessing bioaccumulation of metal. Some exceptions are brain tissue, an important accumulation site for methyl mercury, and bone, a useful tissue for monitoring long-term accumulation of lead. Additionally, blood lead concentrations are used to assess recent exposures and also comprise very useful supporting information when blood delta-ALAD measurements (see section 7.2.2.1.1) are made. If trophic transfers of metals are of interest, whole body concentrations may be important. In plants, roots typically accumulate the highest concentrations of soil- or sediment-borne metals. In the context of trophic transfers, other plant parts may be more important.

7.2.1.1.3 __Methods.__ Most trace metals bioaccumulate and lend themselves readily to direct measurement. Atomic absorption spectroscopy (AAS) has been the method of choice for most metals, and standard methods for AAS analyses in biological media are readily available. More recently, inductively-coupled plasma (ICP) spectroscopy has received considerable attention and is used by some laboratories for routine analyses. Neutron activation analysis (NAA) provides another methodology that is very sensitive for some elements. However, NAA is very expensive and has limited availability; therefore, it is not recommended for routine monitoring of the nature covered by this document. Unlike AAS, ICP and NAA have the capability of simultaneous, multi-element sample analysis, which is often important for environmental monitoring. ICP, however, is not as sensitive a technique for many metals as AAS (particularly flameless AAS). While AAS and ICP involve rather sophisticated instrumentation, trace metal analysis is not inherently difficult, and many laboratories are able to produce reliable data. Generally, trace metal analysis is considerably less time and labor intensive than organic analysis; hundreds of samples can be analyzed in a week.

Van Loon (1985) is an excellent reference covering sample collection and preparation as well as AAS and ICP analyses for trace metals. Sample contamination is a major concern in trace metal analysis. Trace metals, as elements, are ubiquitous and great care must be taken to avoid contamination during sampling, tissue dissection, ashing, and dissolution. Van Loon (1985) describes appropriate precautions for avoiding contamination at these various stages of metal analysis.

7.2.1.1.4 Data Interpretation. There is an extensive amount of literature on trace metal concentrations in a wide variety of organisms. This literature can be very useful for distinguishing between normal (i.e., background) and elevated concentrations of metals. It is important to bear in mind, however, that a number of factors other than environmental concentrations of bioavailable metals influence tissue concentrations within a given species. These factors include season of the year, nutrition, genetic variability among populations, etc. Therefore, one reliable approach for interpreting metal concentrations observed at a waste site is generally to compare the data to those observed in the same species from a nearby reference site known to be minimally contaminated with the metal(s) of interest. Another approach may be a gradient analysis from the source of contamination.

7.2.1.1.5 QA/QC Considerations. Trace metal analysis is sufficiently routine in that standardized QA/QC procedures are followed by most laboratories performing these analyses. These procedures include analysis of National Bureau of Standards reference materials (including bovine liver and orchard leaves), standard additions (spikes), routine analyses of blanks, and inter-laboratory comparisons. A very important consideration here is sample contamination. Since metals of interest as contaminants are also naturally-occurring elements, trace metal analysis is much more prone to artifactual errors due to contamination than organic analysis. Sampling and dissecting equipment must be carefully selected and cleaned, samples carefully handled and stored, and the most metal-free reagents practical employed in sample digestion and analysis. The possibility of metal contamination of reagents, particularly digesting acids, must be scrupulously checked and accounted for with appropriate blanks. See Van Loon (1985) for discussions of this critical topic.

7.2.1.1.6 Case Studies. Many reports concerning trace metal residues in free-living organisms have been published, and many were motivated by concerns of environmental contamination by metals. Informative examples comprising a diverse array of organisms include: Smith and Rongstad (1982) - small mammals; Beyer and Moore (1980) - terrestrial insects and plants; DiGiulio and Scanlon (1984) - waterfowl; Murphy et al. (1978) - fish; and Popham and D'Auria (1983) - bivalves.

### 7.2.1.2 Class I Methods: Organic Chemicals

7.2.1.2.1 Persistence. The issue of persistence is considerably more complex in assessing exposure to organic chemicals than metals. Persistence can be viewed as a gradient from very persistent to rapidly metabolized or excreted. For relatively persistent compounds (including many chlorinated hydrocarbons), direct measures of the parent compound are typically most appropriate. For rapidly metabolized compounds such as organophosphates, indirect measures such as cholinesterases (see Section 7.2.2.2.1) are often more appropriate. For intermediate compounds (such as polycyclic aromatic hydrocarbons), measures of reasonably stable metabolites (see below) can be useful. Unfortunately, for many organics occurring at waste sites (many solvents, for example), limited information concerning persistence and metabolism is available. In these cases, expert opinion should be sought concerning the most appropriate approach. Frequently, the analysis of the parent compound will at least provide information concerning recent exposures.

7.2.1.2.2 Species and Tissue Selections. Questions concerning species and tissues to monitor are more complex for organic compounds than for metals. Site-specific characteristics and the particular questions being asked (trophic transfers, for example) will direct decisions regarding species and tissue selection. In addition to some trace metals, some common organic chemicals such as many organohalogens biomagnify (for example, see Niethammer et al. 1984). For organic chemicals, however, biomagnification appears to be the exception rather than the rule. When sampling an organic chemical that does biomagnify, animals that represent higher trophic levels may be most appropriate for analyses of tissue residues. Liver tissues (or hepatopancreas in many invertebrates) is generally most appropriate for samples. For persistent lipophilic compounds, fatty tissues (such as subcutaneous fat, kidney fat, or brain) are often appropriate. Using bile for polycyclic aromatic hydrocarbon (PAH) metabolites is discussed in section 7.2.1.2.3. In plants, roots often display the greatest concentrations, although in many cases (such as with more volatile compounds), leaves may be more appropriate.

7.2.1.2.3 Methods. The number of organic compounds likely to be encountered at hazardous waste sites is far larger than the number of trace metals, and a far greater number of techniques are available for separating and analyzing organic compounds than metals in biological media. Gas chromatography (GC), GC linked to mass spectroscopy (GC/MS), and high performance liquid chromatography (HPLC) are the most commonly used analytical techniques. However, techniques for organic analysis are far less standardized than is the case for metal analysis. Moye (1981), Natusch and Hopke (1983), and MacLeod et al. (1985) are useful references regarding sample handling, preparation, and analytical procedures. However, diverse techniques are available in this field and are being developed for many compounds. Perhaps the best approach is to secure the services of a very reliable laboratory equipped to perform the specific analysis required.

A relatively new technique that shows considerable promise for routine monitoring of exposure to PAHs in vertebrates is described by Krahn et al. (1984). PAHs are metabolized rather rapidly by vertebrates, and tissue residues of parent compounds are not reliable as indices of exposure to this important group of contaminants. Krahn et al. (1984) uses HPLC linked to a fluorescence detector to estimate concentrations of PAH metabolites in bile. Different fluorescence wavelength pairs are used to measure metabolites of different PAHs (such as naphthalene, phenanthrene, and benzo[a]pyrene). Bile metabolites also provide a useful approach for determining exposure to chlorinated phenolics (Oikari and Anas 1985).

Because applicable techniques are highly variable, it is difficult to estimate the time and labor required for organic analyses. Many compounds can be measured routinely using relatively straightforward GC techniques; others require considerably more sophisticated MS analyses. Generally, organic analyses are considerably more time and labor intensive than metal analyses.

7.2.1.2.4 Data Interpretation. The bulk of the discussion of data interpretation for trace metals data (see section 7.2.1.1.4) applies to organics as well. However, the literature dealing with data interpretation is less extensive for organics than for trace metals. On the other hand, in contrast to trace metals, most organic contaminants are not naturally occurring compounds, which somewhat simplifies data interpretation. Nevertheless, the best approach for assessing the impacts of a particular waste site on tissue burdens of organic contaminants is again the simultaneous collection of analogous data from a nearby reference site.

7.2.1.2.5 QA/QC Considerations. Due to the diversity and rapid evolution of techniques applicable to environmental organic analysis, QA/QC procedures are highly variable. In the context of monitoring at waste sites, these analyses are generally performed under contract, and the contract initiator is strongly urged to carefully select reputable laboratories with documented compliance to appropriate QA/QC procedures.

7.2.1.2.6 Case Studies. As with trace metals, there is an extensive amount of literature concerning residues of many organic compounds in environmentally-exposed organisms. Examples include Niethammer et al. (1984) - various organochlorines; Flickinger et al. (1980, 1984) - organophosphorous compounds and carbamates; Krahn et al. (1986) - bile metabolites of PAHs; and Oikari and Kunnamo-Ojala (1987) - bile metabolites of chlorinated phenolics and resin acids (using caged fish).

### 7.2.2 Indirect Biomarkers for Exposure

### 7.2.2.1 Class I and Class II Methods: Trace Metals

Given the propensity of metals to bioaccumulate as well as the availability of sensitive and accurate techniques for their routine detection in biological samples, indirect indices for exposure to metals are generally not necessary. However, two biomarkers, delta-aminolevulinic acid dehydrase (delta-ALAD) and metal binding protein, discussed in the following subsections, have received considerable attention and may be useful in some cases.

#### 7.2.2.1.1 Class I Methods: Delta-ALAD.

(A) **Species and Tissue Selection**. Delta-ALAD measurements are typically performed in red blood cells, which allows for non-destructive sampling, but which also limits application of the technique to vertebrates. However, it can be adapted for other species and tissues. Regarding other aspects of species selection, the discussions under 7.2.1.1.1 and 7.2.1.1.2 apply. Species and tissue selection for delta-ALAD assays for exposure to lead should be guided by recognition that lead typically does not biomagnify and is typically highly associated with soil/sediment compartments of ecosystems.

(B) **Methods.** The enzyme delta-ALAD catalyzes a reaction involved in heme synthesis and is very sensitive to inhibition by inorganic lead (Goyer 1986), but is relatively insensitive to alkylated lead (Grandjean and Nielsen 1979). Its activity in blood has been used extensively as an index for exposure to inorganic lead in humans and free-living animals. This technique has the advantages over direct lead measurements of being cheaper, simpler, and less subject to the often serious problem of lead contamination of samples. In the context of hazardous waste sites, however, lead is often likely to be one among several metals of interest, and direct multi-element analyses generally will be preferable. If lead is of particular interest, delta-ALAD determinations may be useful.

Burch and Siegel (1971) is considered the standard method for this technique. The technique employs a quite simple, rapid spectrophotometric assay that most biochemical laboratories can readily implement.

(C) Data Interpretation. While typically used as an index of lead exposure, delta-ALAD activities can also provide information concerning sublethal stress due to lead. The inhibition of this enzyme is believed to be an important mechanism underlying lead toxicity (Goyer 1986). However, delta-ALAD activities in the blood of some species, including mammals, have no apparent physiological function, and inhibitions without accompanying deficits (i.e., hemoglobinemia) may occur (Posner 1977).

Blood lead--delta-ALAD relationships, which typically display marked inverse correlations, have been described for a number of species. For informative discussions concerning mammals, birds, and fish, see Hernberg et al. (1970), Dieter and Finley (1979), and Hodson et al. (1979), respectively. Again, however, the best approach for evaluating delta-ALAD data from a given waste site is to employ parallel studies of a neighboring reference site.

(D) QA/QC Considerations. While this approach is not as prone to lead contamination as direct lead analysis, similar precautions must be taken to avoid sample contamination by this ubiquitous metal. For many common species of fish and wildlife, the literature provides baseline delta-ALAD activities that provide a useful check for the performing laboratory.

(E) Case Studies. Excellent examples of the use of delta-ALAD for monitoring for lead exposure in feral animals include: Mouw et al. (1975) - rats; Dieter (1979) - ducks; Kendall and Scanlon (1982) - pigeons; and Hodson et al. (1980) - fish.

7.2.2.1.2 Class II Methods: Metal-Binding Proteins. A number of metals, notably cadmium, copper, mercury and zinc, induce the synthesis of certain low molecular weight metal-binding proteins in a variety of vertebrate and invertebrate species. Certainly the best understood proteins of this group are the metallothioneins. Measures of these proteins have been suggested as useful markers for exposure to certain trace metals or metal mixtures. Such measures, coupled with measurements of metals and metal complexes (for example, complexes including both low and high molecular weight proteins, the latter likely including "target" enzymes), may provide powerful tools for understanding the biological significance of cases of metal contamination.

This approach is presently not sufficiently developed to be recommended as a routine biomonitoring tool. Sufficient understanding of the basic functions of metallothioneins under normal conditions; as well as an understanding of the effects of environmental variables such as season, temperature, and nutrient availability on the metabolism of metal-binding proteins in appropriate indicator species; has not yet been achieved. Additionally, the role of metallothioneins as an adaptive response to metal contamination should be clarified. However, this topic comprises an area of intense research about which those concerned with metal contamination should stay abreast. Furthermore, investigators dealing with metal-contaminated sites who desire in-depth information concerning physiological effects can benefit from presently available approaches.

An excellent reference describing this approach, including a review of specific techniques, is Engel and Roesijadi (1987). Very interesting reports demonstrating the potential utility of vertebrate hepatic metallothionein as a biomarker include Brown et al. (1977), Osborn (1978), and Roch et al. (1982). Since this approach is not recommended as a routine biomarker, it will not be described in greater detail here.

### 7.2.2.2 Class I and Class II Methods: Organic Chemicals

The rapid metabolism of some organics compels a greater need for indirect indices of exposure for these compounds than for metals and persistent organics. Two such indices -- cholinesterases and "drug-metabolizing" enzymes -- have received considerable attention and can provide useful biomarkers.

#### 7.2.2.2.1 Class I Methods: Cholinesterases.

(A) Species and Tissue Selection. This biomarker is applicable to a wide variety of vertebrates and invertebrates, and species selection will likely vary with site characteristics. An important consideration is the generally short half-lives of organophosphorous compounds and carbamates in the environment and in biological tissues. Therefore, the best test species are animals that are likely to be exposed (either directly or through ingestion of contaminated food) soon after these compounds are introduced into the environment.

Use of brain tissue is considered the most reliable approach for determining true acetylcholinesterase activity; inhibition here most closely correlates with other toxic effects, including mortality. However, plasma activities of cholinesterase can also be very useful in vertebrates when non-destructive sampling is desired.

(B) Methods. The cholinesterases are enzymes that are very sensitive to inhibition by organophosphorous (OP) and carbamate compounds; this inhibition underlies the neurotoxicities of these compounds, which include many common insecticides (Murphy 1986). Measures of these enzymes -- acetylcholinestersae (ACh-ase) in brain tissue and butylcholinesterase in plasma -- have been used extensively for monitoring exposure as well as sublethal and lethal effects in a variety of vertebrates and invertebrates. This approach has generally been very successful for OPs, but less so for carbamates. This difference is due to the reversibility of inhibition by carbamates, in contrast to the essentially irreversible nature of OP inhibition. This technique is well refined and currently exists as a powerful tool to monitor both exposure and effects of OPs in a variety of animals. This is fortunate since OPs represent a group of rapidly metabolized organics for which direct analyses can sometimes be difficult.

Ellman et al. (1961) is a generally cited reference describing the cholinesterase assay that is currently undergoing the ASTM standardization process. Hill and Fleming (1982) provide an excellent reference describing the use of this assay in the context of field monitoring. Cholinesterase activity assays are quite straightforward and rapid, and are readily performed by most laboratories equipped for routine biochemical analyses.

(C) Data Interpretation. Relationships among tissue or media concentrations of OPs and carbamates, cholinesterase activities, and toxic effects (particularly mortality) have been described for a number of species (Ludke et al. 1975; Hall and Clark 1982; Rattner and Hoffman 1984; Habig et al. 1986). Therefore, there is extensive literature available that is useful for interpreting cholinesterase activity data in a variety of species. For monitoring avian and fish exposures to these compounds, greater than 20% inhibition of ACh-ase activity has been used as an index for significant exposures and greater than 50% inhibition as indicative of lethal exposures (Holland et al. 1967; Ludke et al. 1975; Tucker and Leitzke 1979). As with most biomarkers, parallel studies of carefully selected reference sites comprise the best approach for interpreting cholinesterase data from a given waste site.

(D) QA/QC Considerations. While cholinesterases are reasonably stable and therefore amenable to biomonitoring, it is very important to treat all samples that are to be compared (such as waste site versus reference site samples) as identically as possible in order to minimize assay variability. The assay itself is relatively straightforward, and routine QA/QC procedures generally employed by reputable laboratories should be adequate. Additionally, the considerable amount of literature available concerning cholinesterase activities in a variety of animals is useful in assessing laboratory performance.

(E) Case Studies. Informative examples of the use of cholinesterase determinations as a biomarker in field studies include: Williams and Sova (1966) - fishes; Zinkl et al. (1979) - birds; and Custer et al. (1985) - various vertebrates.

7.2.2.2.2 Class II Methods: Mixed-Function Oxidase Activities. The study of enzymes involved in the metabolism of lipophilic organic substrates in a wide variety of animals has probably received more attention than any other biochemical response-related topic in this field. These enzyme systems are often referred to as drug or xenobiotic metabolizing systems, although endogenous compounds (such as steroids) may also serve as substrates. These systems comprise a diverse array of enzymes and are often divided into two groups designated "phase one" and "phase two" enzymes (Sipes and Gandolfi 1986). Phase one enzymes typically catalyze the introduction of a polar reactive group (such as -OH) onto the substrate. These reactions generally increase water solubility of the substrate, but their key function is to add or expose functional groups. In phase two reactions, an endogenous, highly water soluble molecule (such as glucuronic acid, glutathione, or sulfate) is covalently linked to the substrate through the functional group resulting from phase one reactions. The conjugated products are generally far more water soluble than the original substrate and thus more readily excreted. Studies of the enzyme systems have focused on liver tissue, although they occur in other organs, including kidneys, lungs/gills, and gonads.

The microsomal mixed-function oxidase (MFO) enzymes occupy a central role in phase one metabolism. These enzymes facilitate oxidations in which one atom of molecular oxygen is reduced to water and the other is incorporated into the substrate (Sipes and Gandolfi 1986). Key components of MFO systems are the terminal oxidases, a group of hemoproteins referred to as the cytochromes P-450. The activities of many MFO-associated enzymes and cytochrome P-450 concentrations are markedly induced in many species by a variety of common environmental pollutants including PAHs, PCBs, and petroleum hydrocarbons (Hodgson et al. 1980; Payne et al. 1987). As with metallothioneins, this feature of induction underlies interest in MFO components as a biomonitoring tool. It should also be noted that while the MFO system may provide tools for biomonitoring, it is also of great inherent toxicological significance. For example, it may provide animals with an adaptive mechanism for coping with some contaminants; alternatively, it can enhance the toxicity of some compounds, as exemplified by the transformation of some procarcinogens to ultimate carcinogens.

While MFO inductions have been used successfully to indicate exposures of animals to relatively low concentrations of contaminants, this approach is not presently recommended as a routine biomarker for hazardous waste sites. As was the case for metallothioneins, MFO activities can provide a very sensitive and useful approach for assessing exposure to inducers in some situations. However, it is premature to draw conclusions regarding their utility for monitoring exposure to many complex mixtures, including types that may occur at waste sites. For example, some metals and solvents (e.g., carbon tetrachloride) can inhibit MFO activities. An important area of research in this area is the study of interactions of MFO inducers and inhibitors.

Payne et al. (1987) is an excellent review concerning the utility of this approach for biomonitoring. This review contains numerous references to techniques pertinent to field applications of MFO components.

## 7.3 BIOMARKERS FOR SUBLETHAL STRESS

Developing useful biomarkers for assessing sublethal stress is currently a very active area of research. However, more biomarkers are developed for exposure assessment than routine biomonitoring. A key approach in developing these biomarkers has been the attempt to adapt techniques developed in various biomedical fields (including toxicology, biochemistry, pathology, and immunology) to various species of ecological concern. Consequently, many potentially useful biomarkers are available and developed. Considerable work is needed, however, to determine which indices show the greatest potential for environmental monitoring and then to adapt these indices from standard mammalian models (rats and mice) to other, diverse species.

Biomarkers of sublethal stress that are considered to be sufficiently well developed for application to waste site assessments are described in the following subsections, which include discussions of "non-specific" and "specific" markers, where specificity refers to particular target tissues or types of compounds.

### 7.3.1 Non-Specific Biomarkers

Again, "non-specific" refers to biomarkers that are not necessarily chemical or tissue/organ specific; although in some cases they may readily be used for specific purposes (for example, histopathology for detecting liver injury).

#### 7.3.1.1 Class I Methods: Histopathology

**7.3.1.1.1** Species and Tissue Selection. Histopathological examinations are generally most useful in a confirmatory role. Due to their relatively high labor and time costs, they are often performed on a subset of organisms being analyzed for simpler markers. Therefore, species and tissue selection is driven largely by factors governing choices for other biomarkers, or by results from preceding biomarker studies.

**7.3.1.1.2** Methods. Routine techniques in histopathology (light microscopy, electron microscopy, and histochemistry) can be adapted for detecting tissue injuries in any selected species. Substantial literature exists describing various pathological effects of a wide variety of chemicals in a large number of species. Generally, histopathology is used to confirm the presence of damaged tissues suggested by biochemical or physiological data, or by the presence of pathogens or chemicals producing established histopathological effects. These techniques are often quite laborious and/or expensive, and their utility in routine biomonitoring may be limited. However, they do provide an important approach for confirming the presence of suspected, key pathologies, such as neoplasms. In this role, they may be an important component of biomonitoring strategies at waste sites. Meyers and Hendricks (1986) is an informative review describing the application of histopathological approaches in ecotoxicology.

Because histopathological techniques vary considerably among different groups of organisms, individuals considering histopathological analyses are strongly encouraged to secure the services of reputable pathologists competent to work with the specific species of interest. It is imperative that the proper tissue collection and fixing techniques appropriate for a particular approach (e.g., light versus electron microscopy, histochemistry) are employed; specific guidance should be obtained from the laboratory that will perform the analyses. Useful references concerning tissue preparation techniques for histopathological studies include: Pearse (1961) - histochemistry; Humason (1962), Lillie (1965), and McDowell and Trump (1976) - general preparative techniques for animals; Miksche and Berlyn (1976) - plant techniques; and Hayat (1986) - preparative techniques for electron microscopy.

7.3.1.1.3 Data Interpretation. Pathologists conducting the analyses should be relied on to interpret results. Although the parallel examination of tissues from reference sites may be unnecessary in some cases (e.g., for histologically well-characterized species), it will often be desirable.

7.3.1.1.4 QA/QC Considerations. Proper and consistent sampling and treatment of samples is of particular concern to the field scientist. Due in part to the importance of histopathology in carcinogenesis testing, QA/QC issues have received considerable attention (Boorman et al. 1985). Reputable laboratories performing histopathological analyses are familiar with these guidelines.

7.3.1.1.5 Case Studies. A few of the many informative studies demonstrating the utility of histopathology in ecotoxicological studies include: Simmons et al. (1988) - complex waste mixtures in mammals; White et al. (1978) - cadmium in birds; Hinton et al. (1988) - progression of neoplasia in fishes; Mix (1983) - neoplasia progression in bivalves; and Godzik (1982) - ultrastructural effects of air pollutants in plants.

#### 7.3.1.2 Class I Methods: Skeletal Abnormalities

7.3.1.2.1 Species Selection. Techniques for determining skeletal abnormalities are generally applicable to any vertebrate species. It is anticipated that this approach will typically be incorporated into more standard laboratory and field studies, which will guide species selection.

7.3.1.2.2 Methods. A number of chemicals, including some trace metals and organics, produce skeletal abnormalities in vertebrates. These effects are generally most pronounced in early life stages, and studies with bird embryos and larval fish have shown these organisms to be very sensitive to a variety of compounds. The techniques for observing these effects appear to be generally uncomplicated and well-researched. This approach appears to have considerable merit as a biomarker for waste site assessments, and several techniques are currently available. With fish, its utility may be limited to adults or to laboratory (or possibly caged, in situ) exposures, since deformed larvae may be rapidly lost to predation in the wild. Bird eggs, however, could be readily sampled in the field and returned to the laboratory for examination. In ecotoxicological studies, this approach has apparently been used mostly with birds and fish. However, the approach could be easily adapted for small mammals.

Gross skeletal deformities are often readily observable with the naked eye. At very early life stages, light microscopy may be required. Although simple visual observations generally are adequate, several other powerful techniques are available when more detailed information is desired. These include radiography (Mayer et al.

1978), measures of mechanical properties of vertebrae (Hamilton et al. 1981), bird embryo skeletal preparations (Karnofsky 1965), and measures of bone components such as collagen (Flanagan and Nichols 1962).

7.3.1.2.3 Data Interpretation. Interpretation of these data is generally not complicated (for example, simple calculations of percent deformities). However, many genetic and environmental factors can give rise to apparently elevated rates of abnormalities, so the parallel study of reference sites is recommended.

7.3.1.2.4 QA/QC Considerations. For the very simple techniques (e.g., visual observations), common sense should suffice. However, for the more involved techniques (such as radiography, collagen content, etc.), the expertise of competent personnel is essential.

7.3.1.2.5 Case Studies. Informative examples of this approach include visually-observable scoliosis in lead-exposed trout (Holcombe et al. 1976), microscopically-observed deformities in mercury-exposed fish (Weis and Weis 1977), altered mechanical properties and biochemical composition in OP-exposed fish (Cleveland and Hamilton 1983), and various deformities in PAH-exposed mallard embryos (Hoffman and Gay 1981). An excellent example of this approach in field monitoring is provided by Hoffman et al. (1988), in which the authors describe various deformities in birds inhabiting an agricultural area (Kesterson NWR, CA) impacted by selenium-enriched drainage waters. Other useful references include Birge and Black (1981), Hoffman and Albers (1984), and McKim (1985).

### 7.3.1.3 Class II Methods: Gas Exchange Measurements in Plants

7.3.1.3.1 **Species and Tissue Selection**. Species selection is likely to be highly site-specific. The instruments used in making gas exchange measurements in plants generally appear adaptable for use with most terrestrial macrophytes and have been used with both leaves and conifer needles.

7.3.1.3.2 **Methods.** Over the past several years, great improvements have been made in portable instruments for gas analysis designed for plant studies. These improved, easy to use instruments allow for rapid, accurate, non-destructive in situ measurements of rates of photosynthesis and respiration, and stomatal conductance. This approach has been recently employed to demonstrate effects of toxicants, including air pollutants, on plants.

Two systems designed for these analyses are described by Atkinson et al. (1986) and Davis et al. (1987). Both utilize portable instruments that monitor carbon dioxide and water vapor concentrations in cuvettes that envelope leaves (or needles of conifers). The instruments include attached microcomputers that essentially convert changes in carbon dioxide and water vapor concentrations to rates of photosynthesis (or respiration) and conductance.

Considerable care must be taken to collect accurate data. The instruments must be carefully and routinely calibrated and environmental variables such as temperature, humidity, and light intensity within the cuvettes must be carefully monitored and controlled. Environmental variables often provide the greatest difficulties in using these instruments to make site comparisons (for example, between waste and reference sites). Supplemental lighting is often used to control this critical variable.

When proper control of potentially confounding variables is achieved, these instruments provide a powerful approach for assessing toxic impacts on plants.

**7.3.1.3.3 Data Interpretation.** The gas exchange responses of plants display high natural variabilities. Therefore, to use this approach to obtain useful data, extra care must be taken to match environmental conditions between waste and reference sites. The literature referenced in section 7.3.1.3.5 of this chapter provides useful discussions relevant to physiological bases of data interpretation.

**7.3.1.3.4 QA/QC Considerations.** The most critical aspects of quality control are discussed in section 7.3.1.3.2. These and other QA/QC considerations are discussed further in the operating manuals provided with the instruments.

**7.3.1.3.5 Case Studies.** The development of portable gas exchange analyzers is fairly recent, and they are just now being used routinely in pollution studies. Informative studies demonstrating their utility for this application include: Coyne and Bingham (1981) - ozone; Wood et al. (1985) - fungicides; and Atkinson et al. (1986) - sulfur dioxide.

### 7.3.2 Specific Biomarkers

The biomarker probably has its greatest appeal and potential in the area of indices specific for particular groups of contaminants or for particular responses (such as genotoxicity). However, only a few specific biomarkers appear to be developed to the point of being available for routine monitoring at waste sites; they are described below. Individuals interested in using the biomarker approach are encouraged to remain informed of additional techniques forthcoming.

#### 7.3.2.1 Class I Methods: Delta-ALAD

This technique was described previously (see section 7.2.2.1.1). While measuring this enzyme in blood is most often used as a biomarker for exposure to lead, it can be considered a very sensitive marker for sublethal stress since its inhibition appears to be a mechanism for lead toxicity (plumbism). However, inhibitions have been observed in the apparent absence of other clinical indications of plumbism. Additionally, the enzyme may have no physiological function in red blood cells. Inhibitions in other tissues, such as liver and brain (Dieter and Finley 1979), have clearer toxicological ramifications. Despite these caveats, delta-ALAD is a very useful tool for monitoring subtle effects of lead exposure in a variety of animals.

#### 7.3.2.2 Class I Methods: Cholinesterases

A number of common waste site chemicals are potent neurotoxins, including trace metals (such as lead and mercury) and various solvents and pesticides. Unfortunately, developed biomarkers for neurotoxins are rarely available for free-living animals. A key exception is the cholinesterases, particularly ACh-ase, which are described in 7.2.2.2.1. Measurements of ACh-ase activity in brain tissue provide a very useful tool for assessing sublethal stress due to OPs, and to a lesser extent, to carbamates. ACh-ase is a "model" biomarker -- its inhibition is the key mode of action for an important group of contaminants. The degree of inhibition can be linked to clinical manifestations of neurotoxicity (altered behavior, tremors, death), and its activity is readily measured in a variety of animals.

#### 7.3.2.3 Class II Methods: DNA Unwinding

Perhaps the single greatest concern related to hazardous waste sites is their potential for releasing carcinogens into the environment. It is in this regard that the biomarker approach in sentinel species may prove most useful. The great concern about elevated rates of neoplasia observed in feral animals inhabiting a number of polluted environments has led to considerable research directed at developing techniques for assessing genotoxicity in free-living animals. Developing this technique has generally involved adaptating existing techniques for genotoxic evaluations in laboratory rodents and humans.

7.3.2.3.1 Species Selection. The DNA unwinding assay appears readily adaptable to vertebrates in general. It may also be applicable to invertebrates and plants, but no reports concerning these organisms have been observed. Species selection among vertebrates will likely be driven largely by site-specific characteristics (for example, which species are available for study, what types of carcinogens occur, etc.). In polluted aquatic systems, benthic animals typically seem most prone to develop tumors (Mix 1986).

7.3.2.3.2 Tissue Selection. The DNA unwinding assay is applicable to any likely target tissue. Typical targets for carcinogens include livers/hepatopancreae, lungs/gills, and gonads. In the fathead minnow experiment described below (Shugart, 1988a), whole fish were used successfully.

7.3.2.3.3 Methods. The alkaline unwinding assay appears to be very applicable to routine monitoring at hazardous waste sites. In this assay, DNA strand breaks due to chemical exposures are quantified by determining the relative proportions of single-stranded and double-stranded DNA following strand separation under carefully defined and controlled conditions of pH and temperature. Shugart (1988a,b) describes this technique for tissues derived from animals exposed in vivo. He has adapted the technique of Daniel et al. (1985) that was developed for human cells in culture. Although Shugart originally developed the technique for fishes, it has also been employed with birds and mammals.

This assay poses no unusual difficulties for laboratories equipped for biochemical studies. With the exception of a fluorometer, only routine reagents and equipment are used, and the assay is far quicker than most alternative probes available for genotoxicity studies in higher organisms. It also appears to be quite sensitive. In a study with benzo[a]pyrene exposure to fathead minnows at 1 µg/L, significant increases in strand breaks were observed (Shugart 1988a). However, no benzo[a]pyrene adducts (a common probe for this chemical) were observed.

7.3.2.3.4 **Data Interpretation.** While the assay is not overly complicated, its development is far too recent for a set of "background" values (of single-strandedness) to be available at this time. Thus, carefully designed studies, including studies at reference sites, appear essential. The biological ramifications of various degrees of single-strandedness are unknown at present; studies should be designed to achieve statistically-based differences for use in interpreting future data.

7.3.2.3.5 **QA/QC Considerations**. The most crucial aspect of this assay appears to be rigorous control of pH, temperature, and incubation time. Laboratories unfamiliar with this relatively new assay will require some effort to gain proficiency.

7.3.2.3.6 **Case Studies.** This technique has only very recently been applied in scenarios applicable to assessments of hazardous waste sites, and these studies have not been published. Shugart (personal communication) has employed the technique to detect DNA damage in fish from systems receiving drainage from waste sites at the Oak Ridge National Laboratory and in cormorants from polluted sites at the

Great Lakes. This laboratory recently observed enhanced DNA unwinding in channel catfish exposed to sediments from Black Rock Harbor, Connecticut (unpublished data); these sediments are enriched in PAHs and PCBs.

## 7.4 REFERENCES

Atkinson, C.J., W.E. Winner, and A.H. Mooney. 1986. A field portable gas-exchange system for measuring carbon dioxide and water vapor exchange rates of leaves during fumigation with $SO_2$. Plant Cell Environ. 9:711-719.

Beyer, W.N., and J. Moore. 1980. Lead residues in eastern tent caterpillars (Malacosoma americanum) and their host plant (Prunus serotina) close to a major highway. Environ. Entomol. 9:10-12.

Birge, W.J., and J.A. Black. 1981. In situ acute/chronic toxicological monitoring of industrial effluents for the NPDES biomonitoringprogram using fish and amphibian embryo-larval stages as test organisms. U.S. Environmental Protection Agency, Office of Water Enforcement and Permits, Report No. OWEP-82-001, Washington, DC.

Boorman, G.A., C.A. Montgomery, Jr., S.L. Eustis, M.J. Wolfe, E.E. McConnell, and J.F. Hardisty. 1985. Quality assurance in pathology for rodentcacinogenicity studies. Pages 345-357. In: Milman, H.A., and E.K. Weisburger, eds. Handbook of Carcinogen Testing. Noyes Publications, Park Ridge, NJ.

Brown, D.A., C.A. Bawden, K.W. Chatel, and T.R. Parsons. 1977. The wildlife community of Iona Island jetty, Vancouver, B.C., and heavy-metal pollution effects. Environ. Conserv. 4:213-216.

Burch, H.B., and A.L. Siegel. 1971. Improved method for measurement of delta-aminolevulinic acid dehydratase activity of humanerythrocytes. Clin. Chem. 17:1038-1041.

Cleveland, L., and S.J. Hamilton. 1983. Toxicity of the organophosphorous defoliant DEF to rainbow trout (Salmo gairdneri) and channel catfish (Ictalurus punctatus). Aquat. Toxicol. 4:341-355.

Coyne, P.I., and G.E. Bingham. 1981. Comparative ozone dose response of gas exchange in a ponderosa pine stand exposed to long-term fumigations. J. Air Pollut. Contr. Assoc. 31:38-41.

Custer, T.W., E. F. Hill, and H.M. Ohlendorf. 1985. Effects on wildlife of ethyl and methyl parathion applied to California rice fields. Calif. Fish Game 71:220-224.

Daniel, F.B., D.L. Haas, and S.M. Pyle. 1985. Quantitation of chemically induced DNA strand breaks in human cells via an alkaline unwinding assay. Anal Biochem. 144:390-402.

Davis, J.E., T.J. Arkebauer, J.M. Norman, and J.R. Brandle. 1987. Rapid field measurement of the assimilation rate versus internal $CO_2$ concentration relationship in green ash (Fraxinus pennsylvanica, Marsh.): The influence of light intensity. Tree Physiol. 3:387-392.

Dieter, M.P. 1979. Blood delta-aminolevulinic acid dehydratase (ALAD) to monitor lead contamination in canvasback ducks (Aythya valisineria). Pages 177-191. In: Animals as Monitors of Environmental Pollutants. National Academy of Sciences, Washington, DC.

Dieter, M.P., and M.T. Finley. 1979. Delta-aminolevulinic acid dehydratase enzyme activity in blood, brain, and liver of lead-dosed ducks. Environ. Res. 19:127-135.

Di Giulio, R.T., and P.F. Scanlon. 1984. Heavy metals in tissues of waterfowl from the Chesapeake Bay, USA. Environ. Pollut. (Ser. A) 35:29-48.

Ellman, G.L., K.D. Courtney, V. Andres, and R.M. Featherstone. 1961. A new and rapid colormetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7:88-95.

Engel, D.W., and G. Roesijadi. 1987. Metallothioneins: A monitoring tool. Pages 421-438 In: Vernberg, W.B., A. Calabrese, F.P. Thurberg, and F.J. Vernberg, eds. Pollution Physiology of Estuarine Organisms. Belle W. Baruch Library in Marine Science No. 17. University of South Carolina Press, Columbia, SC.

Flanagan, B., and G. Nichols. 1962. Metabolic studies of bone in vitro. IV. Collagen biosynthesis by surviving bone fragments in vitro. J. Biol. Chem. 237:3686-3692.

Flickinger, E.L., K.A. King, W.F. Stout, and M.M. Mohn. 1980. Wildlife hazards from furadan 3G applications to rice in Texas. J. Wildl. Manage. 44:190-197.

Flickinger, E.L., D.H. White, C.A. Mitchell, and T.G. Lamont. 1984. Monocrotophos and dicrotophos residues in birds as a result of misuse of organophosphates in Matagorda County, Texas. J. Assoc. Off. Anal. Chem. 67:827-828.

Godzik, S. 1982. The scanning and transmission electron microscopes in use of plants as bioindicators. Pages 79-84. In: Steubing, L. and H.J. Jager, eds. Monitoring of Air Pollutants by Plants: Methods and Problems. Dr W Junk Publishers, The Hague.

Goyer, R.A. 1986. Toxic effects of metals. Pages 582-635. In: Klaassen, C.D., M.O. Amdur, and J. Doull, eds. Casarett and Doull's Toxicology: The Basic Science of Poisons. Macmillan Publishing Co., New York, NY.

Grandjean, P. and T. Nielsen. 1979. Organo-lead compounds: Environmental health aspects. Residue Rev. 72:97-148.

Habig, C., R.T. Di Giulio, A.A. Nomeir, and M.B. Abou-Donia. 1986. Comparative toxicity, cholinergic effects, and tissue levels of S,S,S,-tri-n-butyl phosphorotrithioate (DEF) to channel catfish (Ictalurus punctatus) and blue crabs (Callinectes sapidus). Aquat. Toxicol. 9:193-206.

Hall, R.J. and D.R. Clark, Jr. 1982. Responses of the iguanid lizard Anoliscarolinensis to four organophosphorous pesticides. Environ. Pollut. (Ser. A) 28:42-52.

Hamilton, S.J., P.M. Mehrle, F.L. Mayer, and J.R. Jones. 1981. Method to evaluate mechanical properties of bone in fish. Trans. Am. Fish. Soc. 110:708-717.

Hayat, M.A. 1986. Basic Techniques for Transmission Electron Microscopy. Academic Press, Inc., Orlando, FL.

Hernberg, S., J. Nikkanen, G. Mellin, and H. Lilius. 1970. Delta-aminolevulinic acid dehydrase as a measure of lead exposure. Arch. Environ. Health. 21:140-145.

Hill, E.F. and W.S. Fleming. 1982. Anti chloresterase poisoning of birds: Field monitoring and diagnosis of acute poisons. Environ. Toxicol. Chem. 1:27-38

Hinton, D.E., J.A. Couch, S.J. Teh, and L.A. Courtney. 1988. Cytological changes during progression of neoplasia in selected fish species. Aquat. Toxicol. 11:77-112.

Hodgson, E., A.P. Kulknari, D.L. Fabacher, and K.M. Robacker. 1980. Induction of hepatic drug metabolizing enzymes in mammals by pesticides: A review. J. Environ. Sci. Health. B15:723-754.

Hodson, P.V., B.R. Blunt, D. Jensen, and S. Morgan. 1979. Effect of fish age on predicted and observed chronic toxicity of lead to rainbow trout in Lake Ontario water. J. Great Lakes Res. 5:84-89.

Hodson, P.V., B.R. Blunt, and D.M. Whittle. 1980. Biochemical monitoring of fish blood as an indicator of biologically available lead. Thalassia Jugosl. 16:389-396.

Hoffman, D.J. and P.H. Albers. 1984. Evaluation of the potential embryotoxicity and teratogenicity of 42 herbicides, insecticides, and petroleum contaminants to mallard eggs. Arch. Environ. Contam. Toxicol. 13:15-27.

Hoffman, D.J., and M.L. Gay. 1981. Embryotoxic effects of benzo[a]pyrene, chrysene, and 7,12-dimethylbenz[a]anthracene in petroleum hydrocarbon mixtures in mallard ducks. J. Toxicol. Environ. Health. 7:775-787.

Hoffman, D.J., H.M. Ohlendorf, and T.W. Aldrich. 1988. Selenium teratogenesis in natural populations of aquatic birds in central California. Arch. Environ. Contam. Toxicol. 17:519-525.

Holcombe, G.W., D.A. Benoit, E.N. Leonard, and J.M. McKim. 1976. Long-term effects of lead exposure on three generations of brook trout (Salvelinus fontinalis). J. Fish Res. Board Can. 33:1731-1741.

Holland, H.T., D.L. Coppage, and P.A. Butler. 1967. Use of fish brain acetylcholinesterase to monitor pollution by organophosphorous pesticides. Bull. Environ. Contam. Toxicol. 2:156-162.

Humason, G.L. 1962. Animal Tissue Techniques. Freeman, San Francisco, CA.

Jernelov, A. 1972. Mercury and food chains. Pages 174-177. In: Hartung, R., and B.D. Dimman, eds. Environmental Mercury Contamination. Ann Arbor Science Publishers, Inc., Ann Arbor, MI.

Karnofsky, D.A. 1965. The chick embryo in drug screening: Survey of teratological effects observed in the 4-day old chick embryo. Pages 185-215. In: Wilson, J.G., and J.K. Warkany, eds. Teratology: Principles and Techniques. University of Chicago Press, Chicago, IL.

Kendall, R.J., and P.F. Scanlon. 1982. Tissue lead concentrations and blood characteristics of rock doves from an urban setting in Virginia. Arch. Environ. Contam. Toxicol. 11:265-268.

Krahn, M.M., M.S. Myers, D.G. Burrows, and D.C. Malins. 1984. Determination of metabolites of xenobiotics in bile of fish from polluted waterways. Xenobiotica 14:633-646.

Krahn, M.M., L.D. Rhodes, M.S. Myers, L.K. Moore, W.D. MacLeod, Jr., and D.C. Malins. 1986. Associations between metabolites of aromatic compounds in bile and the occurrence of hepatic lesions in English sole (Parophrys vetulus) from Puget Sound, Washington. Arch. Environ. Contam. Toxicol. 15:61-67.

Lillie, R.D. 1965. Histopathologic Technique and Practical Histochemistry. McGraw-Hill, New York, NY.

Ludke, J.L., E.F. Hill, and M.P. Dieter. 1975. Cholinesterase (ChE) response and related mortality among birds fed ChE inhibitors. Arch. Environ. Contam. Toxicol. 3:1-21.

MacLeod, W.D., Jr., D.W. Brown, A.S. Friedman, D. Burrows, O. Maynes, R. Pearce, C. Wigren, and R. Bogar. 1985. Standard Analytical Procedures of the NOAA National Analytical Facility 1984-5: Extractable Toxic Organic Compounds. NOAA Tech. Memo. NMFS, F/NWC6-64. 100 pp.

Mathis, B.J., T.F. Cummings, M. Gower, M. Taylor and C. King. 1979. Dynamics of manganese, cadmium, and lead in experimental power plant ponds. Hydrobiologia 67:197-206.

Mayer, F.L., P.M. Mehrle, and P.L. Crutcher. 1978. Interactions of toxaphene and vitamin C in channel catfish. Trans. Am. Fish. Soc. 107:326-333.

McDowell, E.M., and B.F. Trump. 1976. Histologic fixation suitable for diagnostic light and electron microscopy. Arch. Pathol. Lab. Med. 100:405-414.

McKim, J.M. 1985. Early life stage toxicity tests. Pages 58-95. In: Rand, G.M., and S.R. Petrocelli, eds. Fundamentals of Aquatic Toxicology: Methods and Application. Hemisphere Publishing Corp., Washington, DC.

Meyers, T.R., and J.D. Hendricks. 1986. Histopathology. Pages 283-331. In: Rand, G.M., and S.R. Petrocelli, eds. Fundamentals of Aquatic Toxicology: Methods and Application. Hemisphere Publishing Corp., Washington, DC.

Miksche, J.P., and G.P. Berlyn. 1976. Botanical Microtechnique and Cytochemistry. Iowa State University Press, Ames, IA.

Mix, M.C. 1983. Haemic neoplasms of bay mussels, Mytilus edulis L. from Oregon: Occurrence, prevalence, seasonality and histopathological progression. J. Fish Dis. 6:239-248.

Mix, M.C. 1986. Canceroous diseases in aquatic animals and their association with environmental pollutants: A critical review. Mar. Environ. Res. 20:1-141.

Mouw, D., K. Kalitis, M. Anver, J. Schwartz, A. Constan, R. Hartung, B. Cohen, and D. Ringler. 1975. Lead: Possible toxicity in urban vs. rural rats. Arch. Environ. Health 30:276-280.

Moye, H.A., ed. 1981. Anaysis of Pesticide Residues. Chemical Analysis Series, Vol. 58. Wiley and Sons, New York, NY.

Murphy, B.R., G.J. Atchison, A.W. McIntosh, and D.J. Kolar. 1978. Cadmium and zinc content of fish from an industrially contaminated lake. J. Fish Biol. 13:327-335.

Murphy, S.D. 1986. Toxic effects of pesticides. Pages 519-581. In: Klaassen, C.D., M.O. Amdur, and J. Doull, eds. Casarett and Doull's Toxicology: The Basic Science of Poisons. Macmillan Publishing Co., New York, NY.

Natusch, D.F.S., and P.K. Hopke, eds. 1983. Analytical Aspects of Environmental Chemistry. Chemical Analysis Series, Vol. 64. Wileyand Sons, New York, NY.

Niethammer, K.R., D.H. White, T.S. Baskett, and M.W. Sayre. 1984. Presence and biomagnification of organochlorine chemical residues in oxbowlakes of northeastern Louisiana. Arch. Environ. Contam. Toxicol. 13:63-74.

Oikari, A., and E. Anas. 1985. Chlorinated phenolics and their conjugates in the bile of trout (Salmo gairdneri) exposed to contaminated waters. Bull. Environ. Contam. Toxicol. 35:802-809.

Oikari, A., and T. Kunnamo-Ojala. 1987. Tracing of xenobiotic contamination in water with the aid of fish bile metabolites: A field study withcaged rainbow trout (Salmo gairdneri). Aquat. Toxicol. 9:327-341.

Osborn, D. 1978. A cadmium and zinc binding protein from the liver and kidney of Fulmaris glacialis, a pelagic North Atlantic seabird. Biochem. Pharmacol. 27:822-824.

Payne, J.F., L.L. Fancey, A.D. Rahimtula, and E.L. Porter. 1987. Review and perspective on the use of mixed-function oxyfenase enzymes in biological monitoring. Comp. Biochem. Physiol. 86C:233-245.

Pearse, A.G.E. 1961. Histochemistry, Theoretical and Applied. Little, Brown, Boston, MA.

Popham, J.D., and J.M. D'Auria. 1983. Combined effect of body size, season, and location on trace element levels in mussels (Mytilus edulis). Arch. Environ. Contam. Toxicol. 12:1-4.

Posner, H.S. 1977. Indices of potential lead hazard. Environ. Health Perspect. 19:261-284.

Rattner, B.A., and D.J. Hoffman. 1984. Comparative toxicity of acephate in laboratory mice, white-footed mice, and meadow voles. Arch. Environ. Contam. Toxicol. 13:483-491.

Roch, M., J.A. McCarter, A.T. Matheson, M.J.R. Clark, and R.W. Olafson. 1982. Hepatic metallothionein in rainbow trout (Salmo gairdneri) as an indicator of metal pollution in the Campbell River system. Can. J. Fish. Aquat. Sci. 39:1596-1601.

Shugart, L. 1988a. An alkaline unwinding assay for the detection of DNA damage in aquatic organisms. Mar. Environ. Res. 24:321-325.

Shugart, L. 1988b. Quantitation of chemically-induced damage to DNA of aquatic organisms by alkaline unwinding assay. Aquat. Tox. In press.

Simmons, J.E., D.M. DeMarini, and E. Berman. 1988. Lethality and hepatotoxicity of complex waste mixtures. Environ. Res. 46:74-85.

Sipes, I.G., and A.J. Gandolfi. 1986. Biotransformation of toxicants. Pages 64-98. In: Klaassen, C.D., M.O. Amdur, and J. Doull, eds. Casarett and Doull's Toxicology: The Basic Science of Poisons. Macmillan Publishing Co., New York, NY.

Smith, G.J., and O.J. Rongstad. 1982. Small mammal heavy metal concentrations from mined and control sites. Environ. Pollut. (Ser. A) 28:121-134.

Tucker, R.K., and J.S. Leitzke. 1979. Comparative toxicology of insecticides for vertebrate wildlife and fish. Pharmac. Ther. 6:167-220.

Van Loon, J.C., ed. 1985. Selected Methods of Trace Metal Analysis: Biological and Environmental Samples. Chemical Analysis Series, Vol. 80. Wiley and Sons, New York, NY.

Weis, P., and J.S. Weis. 1977. Methylmercury teratogenesis in the killifish, Fundulus heteroclitus. Teratology 16:321-324.

White, D.H., N.T. Fiwley, and J.F. Ferrel. 1978. Histopathological effects of dietary cadmium on kidneys and testes of mallard ducks. J. Toxicol. Environ. Health. 4:551-558.

Williams, A.K., and C.R. Sova. 1966. Acetylcholinesterase levels in brains of fishes from polluted water. Bull. Environ. Contam. Toxicol. 1:198-204.

Wood, B.W., J.A. Payne, and T.R. Gottwald. 1985. Inhibition of photosynthesis in pecan leaves by fungicides. Plant Dis. 69:997-998.

Zinkl, J.G., C.J. Henny, and P.J. Shea. 1979. Brain cholinesterase activities of passerine birds in forests sprayed with cholinesterase inhibitors. Pages 356-365. In: Animals as Monitors of Environmental Pollutants, National Academy of Science, Washington, DC.

# CHAPTER 8

# FIELD ASSESSMENTS

By

Lawrence A. Kapustka, U.S. Environmental Protection Agency,
Environmental Research Laboratory, Corvallis, OR.

Thomas W. LaPoint and James F. Fairchild, U.S. Fish and Wildlife Service,
National Fisheries Contaminant Research Center, Columbia, MD.

Karen McBee, Department of Zoology,
Oklahoma State University, Stillwater, OK.

Jerry J. Bromenshenk, Division of Biological Science,
University of Montana, Missoula, MT.

## 8.1 INTRODUCTION -- Lawrence A. Kapustka

Detailed assessments of ecological effects involves some measurement of structural and functional relationships of biota spanning the range of individuals to ecosystems. This is the role of aquatic and terrestrial field surveys in hazardous waste site (HWS) assessments. Ecological field surveys are a definitive way to establish that adverse ecological effects have occurred. Data generated from field surveys are evaluated with data derived from chemical analysis and toxicity testing to provide an integrated ecological assessment of the HWS.

There are several distinct reasons for implementing field surveys as assessment tools at an HWS. First, indigenous organisms serve as continuous monitors of environmental quality by integrating potentially wide fluctuations in contaminant exposure. Second, an accurate field assessment of natural populations directly measures adverse effects; thus, extrapolations from laboratory data are not necessary for interspecies sensitivity, environmental variation, pulsed dosing, chemical interaction (additivity, antagonism, or synergism), or bioavailability. Third, results of the assessment of indigenous populations are directly interpretable, since effects are quantified on the resources actually at risk. Fourth, the results of assessments of effects on indigenous populations are easily understood by managers, regulators, and the general public. Thus, field surveys of indigenous organisms are useful for identifying flora and fauna at risk as well as for direct quantification of environmental effects.

Hazardous waste sites present unique constraints of access and risk to environmental scientists. Some sites, because of extremely limited size and/or the nature of habitat disturbance, do not pose substantive ecological concerns. At other sites, however, ecological field assessments can play a major role in defining the nature of the problems associated with the site. Furthermore, the ecological assessment should be considered as a benchmark for evaluating the success of any remedial actions.

This chapter on field assessment focuses on sampling strategies that have been selected for HWS assessments. The emphasis is on data acquisition. Given the temporal limitations on data collection that often pertain to HWSs, it is important to emphasize the influence that such sampling constraints may have on the uncertainty associated with the resulting data. One-time sampling efforts almost always underestimate species richness because ephemeral populations are easily missed and quantitative estimates derived from these static samples underestimate the dynamics of the site.

Only passing comments on data reduction are provided in this chapter. None of the ecological divisions addressed here have universally accepted, consistently used indices that can be used to condense the information into simple terms. Professional expertise is usually required to interpret patterns of species assemblages and populations.

## 8.2 AQUATIC SURVEYS -- Thomas W. LaPoint and James F. Fairchild

### 8.2.1 Introduction

This section describes various methods and endpoints that can be used in field surveys of aquatic organisms. Methods described consist of accepted, published approaches (Class I) commonly used to monitor periphyton, plankton, macroinvertebrates, and fish in a variety of aquatic habitats. The methods are briefly described, along with common precautions and limitations relating to their use. Endpoints consist primarily of direct and derived measures of population and community structure, such as relative abundance, species richness, and indices of community organization. Sources of comprehensive, detailed information are provided in the form of references for each topic. Comprehensive documents useful in conducting field surveys include APHA (1985), U.S. EPA (1973), Platts et al. (1983), U.S. EPA (1987), ASTM (1987a), and Plafkin et al. (1988).

### 8.2.2 Endpoints

Aquatic field surveys for the biological effects of contaminants associated with an HWS involve the measurement or monitoring of population and community structure. Structural endpoints include relative abundance, species richness, community organization (diversity, evenness, similarity, guild structure, and presence or absence of indicator species), and biomass. Functional endpoints, such as cellular metabolism, individual or population growth rates, and rates of material or nutrient transfer (e.g., primary production, organic decomposition, or nutrient cycling) are less commonly measured. Functional measurements are important in interpreting the ramifications of an observed change in population or community structure. However, functional measures are difficult to interpret in the absence of structural information and frequently require considerable time, equipment, and expertise. In addition, procedures for functional assessments have not been standardized and require considerable understanding of the system and processes involved. Functional measures may therefore be limited in application to the assessment of HWS effects unless conducted in a research framework.

### 8.2.2.1 Species Richness and Relative Abundance

Species richness (the number of species in a community) and relative abundances (the number of individuals in any given species compared to the total number of individuals in the community) are structural endpoints commonly measured in field surveys of periphyton, plankton, macroinvertebrates, and fish. Estimates of relative abundance or species richness can yield readily interpretable information on the degree of contamination of an aquatic habitat (Sheehan and Winner 1984; Lamberti and Resh 1985; Hellawell 1986). Loss of a particular species from an ecosystem can be critical when that species plays an important role in community or ecosystem functions such as predation (Paine 1969) or grazing (Giesy et al. 1979).

Measures of species richness and relative abundance are taken by sampling known substrate areas or water volumes. Richness measures have not always been taken to the species level, especially in monitoring invertebrate communities. Taxonomic, fiscal, and time constraints have often predicated the need for rapid bioassessment (e.g., Hilsenhoff 1988; Plafkin et al. 1988) involving taxonomic identifications only to family and genus. It is probable that such identifications at lower levels of resolution result in some loss of sensitivity to HWS effects.

### 8.2.2.2 Biomass

Biomass measurements, defined as the mass of tissue present in an individual, population, or community at a given time, is another potential structural endpoint. Biomass can be directly measured gravimetrically on wet or dry tissue. However, direct measurement of biomass of individuals is often time-consuming, and direct measurements of individual biomass of phytoplankton, zooplankton, or macroinvertebrates are impossible due to analytical insensitivity. Thus, biomass is estimated gravimetrically by using pooled samples of individuals or by an indirect method. Indirect estimates of invertebrate or fish biomass can be indirectly estimated by using empirical or published length:weight regressions. However, biomass measurements on these trophic groups are not commonly performed in routine field surveys.

Biomass of periphyton communities is commonly measured. Measurements of phytoplankton or periphyton biomass can be estimated on the basis of ash-free dry mass (AFDM) or chlorophyll a content (APHA 1985). Chlorophyll measurements are performed by solvent extraction, followed by spectrophotometry or fluorometry (APHA 1985).

### 8.2.2.3 Indicator Species

The presence or absence of "indicator species" is commonly used to assess adverse effects to ecological communities (Karr et al. 1986; Hilsenhoff 1988; Plafkin et al. 1988). The concept was originally derived from the saprobian system, in which certain species and groups were found to generally characterize stream and river reaches subject to organic wastewaters; increasing anthropogenic organic matter in aquatic habitats serves to fill the energy requirements of "tolerant" species, while reducing the numbers of "sensitive" species that respond negatively to competition, predation, or decreased dissolved oxygen (Kolkwitz and Marsson 1902; Gaufin 1958; Sheehan 1984).

Experience has shown that the indicator species concept lacks broad applicability to all types of pollution. Sheehan (1984) indicated that communities do not respond to organic wastes (e.g., sewage) in the same way they respond to toxic chemicals. Organic sewage stimulates certain species by increasing their food supply; other species consequently diminish as a result of interspecific interactions. Toxic chemicals, on the other hand, tend to affect all members of a community. Furthermore, species selection may occur in aquatic habitats that are chronically polluted with low levels of contaminants over sufficiently long periods. In such instances, certain species that ordinarily appear to be quite "sensitive" may seem to be "tolerant" due to decreases in predation or competitive pressures (Hersh and Crumpton 1987).

However, the indicator species concept can be applied to the assessment of ecological effects if enough care is taken to limit the breadth of its application. Some species may be found upstream from the HWS or in habitats known to be unaffected by HWS seepages. The indicator species concept has been applied in assessment techniques for hazardous effluents (Courtemanch and Davies 1987) and metals (Sheehan and Winner 1984). In a similar approach, although at lower taxonomic resolution, the total numbers of insects in the orders Ephemeroptera, Plecoptera, and Trichoptera are counted and referred to as the number of "EPTs" (Hilsenhoff 1988; Plafkin et al. 1988). Typically, these three orders are sensitive to metals and other inorganic contaminants and, thus, provide an index of effect. Karr (1981) applied the indicator species concept in the Index of Biotic Integrity (IBI), in which fish community composition is used as a measurement of environmental quality (see section 8.2.3.4 on fish).

### 8.2.2.4 Indices

Biological indices can be used to mathematically reduce taxonomic information to a single number or index, to simplify data for interpretation or presentation. Indices derived from direct measures of the presence of taxa have been extensively developed, reviewed, and critiqued (Sheehan 1984; Hellawell 1986). Indices can be classified among several types: evenness (measuring how equitably individuals in a community are distributed among the taxa present); diversity (calculating the abundance of individuals in one taxon relative to the total abundance of individuals in all other taxa); similarity (comparing likeness of community composition between two sites); and biotic indices (examining the environmental tolerances or requirements of individual species or groups).

Although indices may aid in data reduction, they should never be divorced from the actual data on species richness and abundance. Relying on a single index such as the Shannon-Weiner Index is sometimes misleading. For example, a few individuals evenly distributed among several species could give a relatively high index of diversity, even though a habitat is grossly polluted. In addition, statistical assumptions of independence, normality, and homogeneity of variance are frequently invalid for these derived, proportional measures. Hence, when indices are used, statistical transformations (e.g., arc-sine) or rank-order statistics (Siegel 1956; Green 1979; Hoaglin et al. 1985) are recommended.

### 8.2.2.5 Guild Structure

Community data generated at the species level can be analyzed according to guild structure. Guilds, or functional feeding groups, are classifications based on the manner in which organisms obtain their food and energy. Invertebrates can be classified among such functional groups as collector-gatherers, piercers, predators, scrapers, and shredders (Merritt and Cummins 1984; Cummins and Wilzbach 1985); and fish can be classified as omnivores, insectivores, and piscivores (Karr et al. 1986). Shifts in community guild structure reflect changes in the trophic-dynamic status of an aquatic ecosystem. For example, contaminant influences from an HWS may eliminate or reduce periphyton and thus concomitantly reduce the relative abundance of scrapers (herbivores) in relation to other invertebrate guilds such as collector-gatherers. Changes may also occur within a guild, such as when a contaminant alters the level of competition between two species that compete for a common resource (Petersen 1986). Generally, the effects must be fairly strong to enable the measurement of changes in guild structure.

### 8.2.3 Methods

#### 8.2.3.1 Periphyton

Periphyton communities sometimes provide sensitive tools with which to detect changes in lotic environments that result from contaminants (Lewis et al. 1986; Stevenson and Lowe 1986; Crossey and LaPoint 1988). Monitoring may involve sampling either natural or standardized substrates. Taxonomic composition and relative abundance of periphyton are more variable on natural substrates than on standardized substrates, although the variance can be reduced by carefully selecting specific microhabitats with similar physical and chemical characteristics such as substrate type, current velocity, depth, and ambient light (see Table 8-1 for methods) (Stevenson and Lowe 1986). On hard substrates, data on algal abundance, biomass, and species composition can be obtained by removing the substrate and by scraping or brushing the flora from a measured area into a container. Alternatively, the desired sampling area can be isolated or enclosed by using a chamber sealed to the substrate with neoprene (or other thick rubberized material), or by using a coring device and removing the scraped material by suction into a vial (Hamala et al. 1981).

Collecting algae from soft sediments is much more laborious, for it involves using vacuum suction to remove the soft organic surficial sediment layer and then sorting through the debris for algae for quantitative counts (Stevenson and Lowe 1986).

Table 8-1. Methods for Measuring Physical and Chemical Variables

| Measurement | Reference |
|---|---|
| Temperature | APHA (1985) |
| Dissolved oxygen | APHA (1985) |
| Alkalinity | APHA (1985) |
| Hardness | APHA (1985) |
| Conductivity | APHA (1985) |
| Nutrients (ammonia, nitrate/nitrite, ortho-phosphate) | APHA (1985) |
| Current velocity | Hamilton and Bergersen (1984) |
| Substrate composition | Platts et al. (1983); Hamilton and Bergersen (1984) |
| Photosynthetically active radiation | Li-Cor (1979) |

Standardized substrates have been applied widely in environmental assessments of periphyton colonization and community organization. Materials used as standardized substrates include granite slabs, plastic strips, tiles, and glass slides. Diatometers, consisting of frosted glass slides placed into a holding frame and immersed in the water, are broadly accepted. Although diatometers are known to be somewhat selective because not all algal taxa colonize the glass surfaces, this disadvantage is offset by gains in sampling convenience and replicability that result from the similarities in surface texture, surface area, colonization time, and microenvironmental conditions. Descriptions of diatometers and methods for their use were given by Gale et al. (1979) and APHA (1985).

After the periphyton sample is obtained from a given sampling area, it may be analyzed for taxonomic composition (cell number, species richness, and relative abundance). Community indices (diversity, community similarity, etc.) can be calculated from the taxonomic data. Standing crop (chlorophyll a or AFDM per unit area) can be determined according to standard and accepted methods (Vollenweider 1974; APHA 1985); an Autotrophic Index (AFDM divided by chlorophyll a, both in $mg/m^2$) can be calculated (APHA 1985) as well as several other productivity-related indices (cf. Crossey and LaPoint 1988). One common caution in conducting algal surveys is that enough cells must be counted to ensure that rare species are quantified. For example, Stevenson and Lowe (1986) recommended counting 200 cells from each sample to ensure complete enumeration of dominants, 500 cells to ensure the inclusion of uncommon taxa, and 1000 cells to adequately record rare species. Alternatively, they suggested that counting be continued until fewer than one new species is encountered for each additional 100 algal cells counted.

Studies of periphyton communities should be supported by additional physical and chemical information that sometimes influences periphyton production and dynamics. It is desirable to collect data on substrate composition, current velocity, temperature, photosynthetically active radiation (PAR), dissolved oxygen, conductivity, alkalinity, hardness, and dissolved nutrients (ortho-phosphate, ammonia, and nitrate/nitrite). Methods for measuring variables are given in Table 8-1. Although the appropriate selection of reference sites should remove sources of covariance, it is important to document these factors for quality assurance and interpretive purposes.

#### 8.2.3.2 Plankton

Many devices are available for sampling plankton, and sampling techniques for phytoplankton and zooplankton are similar. The choice of an individual sampling technique, sample size, and sample numbers, whether for zooplankton or phytoplankton, will depend upon the characteristics of the aquatic habitat (in terms of depth, density of organisms, and spatial variation). Samplers are broadly categorized into four types: closing samplers, traps, pumps, and nets (De Bernardi 1984; APHA 1985; ASTM 1987b-d). DeBernardi (1984) published a schematic diagram for choosing among different zooplankton sampling methods for different types of habitats and samples.

Closing samplers (bottles or tubes) are lowered into the water to a particular depth and closed with a drop-weight messenger; examples are the Van Dorn and Kemmerer models (DeBernardi 1984; ASTM 1987b). These samplers take a quantitative sample of water at a chosen depth, collecting all forms of nannoplankton and ultraplankton. Closing samplers can be obtained or constructed for many different volumetric requirements. A series of closing-bottle samplers can be vertically arranged to sample simultaneously at multiple depths, to determine plankton stratification. In shallow water, plankton stratification can be mechanically integrated by using a depth-integrating column sampler (cf. Bloesch 1988). These types of closing samplers capture a known volume of water by extending a tube through the water column from the surface to the bottom. The water cores sampled typically vary in length (from one to several meters long) and diameter (from one to several centimeters), depending upon the experimental conditions. Because these samplers integrate plankton distributions throughout the water column, they yield no useful information on plankton stratification.

Traps such as the Juday, Patalas, and Schindler types, which have been used for zooplankton sampling (DeBernardi 1984), are basically large closing-type samplers that can be lowered into the water to sample water volumes of 10 to 30 L. The large size of the traps is thought to reduce avoidance by the more agile zooplankters, such as adult copepods, and to increase sampling efficiency for potentially rare species. The maneuverability of relatively large traps can make them somewhat more difficult to maneuver than other samplers.

Various pumps have also be been applied in plankton sampling (DeBernardi 1984; ASTM 1987c). Pumps can be either motorized or hand-operated; but motorized pumps are preferred because they provide uniform delivery rates. Both submersible and boat-mounted pumps have been used. Sample size is determined by using a flowmeter or by collecting the sample in a calibrated container. Pumps can be used to take either discrete samples at a particular depth or integrated samples over a range of depths. They allow a researcher to easily increase or decrease sample size by changing the pumping time or pumping rate, and are amenable for use in a variety of aquatic habitats. However, pumps have been criticized as being expensive and somewhat bulky. In addition, care must be taken to insure that organisms are not damaged by the pumping device, and that pumps are adequately flushed to prevent cross-contamination of samples.

Conical nets are also commonly used for quantitative zooplankton sampling (DeBernardi 1984; ASTM 1987d). Pore sizes of the nets typically range from 60 to 80 µm. Because a mesh of this size does not retain ultraplankton and nannoplankton, net samples for phytoplankton are qualitative. Net samplers are towed with a rope for a desired distance or time. Sample size is determined by a flowmeter, the distance towed, or other estimate of sample volume (such as distance multiplied by aperture area). Net samples can be taken in either vertical or horizontal tows, depending on the desired sampling strata. Some net samplers, such as the Birge closing net, have a closure feature that enables the operator to sample discrete depths or distance.

Collected samples can be isolated or concentrated by using various techniques. Both phytoplankton and zooplankton can be isolated using settling chambers (APHA 1985). Zooplankton can be isolated by using a net or other sieving device of a mesh size compatible with the original collection method. After isolation, plankton samples must be preserved (APHA 1985) and stored for taxonomic identification. Species richness, relative abundance, and community indices can be determined from the taxonomic data.

#### 8.2.3.3 Macroinvertebrates

Benthic invertebrates are the most common fauna used in ecological assessments of contaminants. Numerous excellent references deal with the collection, identification, and analysis of benthic invertebrate populations (e.g., Southwood 1978; Downing 1984; Merritt and Cummins 1984; Peckarsky 1984; APHA 1985; ASTM 1987e-i). Macroinvertebrates are operationally defined as the invertebrates retained by screens of mesh size greater than 0.2 mm (Hynes 1971). Larger mesh sizes (such as the 0.595 mm, U.S. Standard No. 30, APHA 1985) have been accepted as standard for routine biomonitoring. Microinvertebrates (rotifers, nematodes, gastrotrichs) may be of ecological interest, but their taxonomy is much less known; consequently, their sampling is not recommended for routine environmental assessments.

A variety of techniques can be used to collect macroinvertebrates from aquatic environments (see Table 8-2 for a summary of macroinvertebrate sampling methods, including time and labor estimates.) In any given contaminant effects study, careful consideration must be given to the comparability of samples among stations. Not only must the type of sampling device be appropriate for the specific taxa and habitat type, but sampling effort (e.g., sample numbers and sample sizes) must be uniform at all stations. As in assessing contaminant effects with periphyton, macroinvertebrates can be collected and quantified by sampling either natural or standardized substrates.

Natural substrates can be sampled with net, grab, core, and vegetation samplers. Hess and Surber samplers are commonly used to collect benthic invertebrate fauna in shallow riffle habitats of streams (ASTM 1987e). These two samplers are similar in that each encloses a defined area (0.1 $m^2$ ) of substrate. Substrate within the confines of the sampler is disturbed and mixed by hand or stake to a depth of 10 cm. Large rocks within the sampled area are manually lifted from the substrate and brushed or scrubbed at the mouth of the sampler to dislodge attached or clinging invertebrates, which are carried downstream into the net by the current; a current velocity of at least 0.05 m/s is required for effective use of the Surber or Hess sampler. Further information on selecting stream-net samplers is given in ASTM (1987f).

Surber and Hess samplers generally do not operate effectively in large rivers, estuaries, lakes, or other habitats with soft substrates because the current needed to dislodge and wash invertebrates into the sampler net is lacking. Furthermore, water that is is too deep flows over the top of the sampler. Consequently, core and grab samplers are used in these habitats. These techniques are further described in a handbook by Lind (1979).

### Table 8-2. Sampling Methods for Macroinvertebrates

| Method | Habitat | Substrate Type | Effort Required[1] |  | Reference |
|---|---|---|---|---|---|
|  |  |  | Persons | Time (hr) |  |
| Hess, Surber | stream riffle (<0.5 m deep) | sand, gravel, cobble | 1 | 0.50 | ASTM (1987e) |
| Ponar grab | rivers, lakes, estuaries | mud, silt, sand, fine gravels | 2 | 0.50 | ASTM (1987i) |
| Ekman grab | stream pools, shallow lakes | mud, silt, sand | 1 | 0.25 | ASTM (1987h) |
| Corers | rivers, lakes | mud, silts | 1-2 | 0.25 | Downing (1984) |
| Sweep net | littoral | vegetation | 1 | 0.25 | Downing (1984) |
| Macan<br>McCauley<br>Minto<br>Wilding | littoral | vegetation | 1 | 0.50 | Downing (1984) |
| Standardized substrates | all | all | 1 | 0.25-1.0[2] | APHA (1985) |

[1] Effort includes time spent in field to collect, sieve, and isolate one sample. Laboratory time required to remove and identify organisms ranges from 1 to 5 hr per sample, depending on taxonomic resolution sought.

[2] Six-week colonization time needed before sample is removed.

Corers, such as the Kajak-Brinkhurst (Downing 1984; APHA 1985) and Phleger (APHA 1985) types, are recommended for soft substrates such as silts or clays. Corers consist of long, open tubes and rely on gravity to penetrate the substrate. Various closure methods are used to seal the tube before it is retrieved from a fixed area of sediment.

Various types of grab samplers are available for sampling macroinvertebrates in different habitats. Extensive descriptions, including discussions of advantages and limitations of the various grab samplers, are given by ASTM (1987g). Grab samplers operate by isolating and removing an area of substrate defined by the area of the open jaws of the apparatus. Choice of a particular type of sampler depends on the type and size of substrate and depth of water in the aquatic habitat. Two of the most popular are the Ekman and Ponar types. Ekman grab samplers (ASTM 1987h; APHA 1985) are useful for sampling relatively shallow habitats containing soft mud and silt in water with little current. One person, using a pole mount or remote messenger, can easily sample the benthos with an Ekman grab sampler from a boat or while wading in shallow water. The grabs are difficult to use on pebbly or rocky bottoms because gravel often impedes jaw closure. Ponar grab samplers (APHA 1985; ASTM 1987i) are used to sample substrates such as sand, gravel, or small rocks in medium to deep rivers, estuaries, and lakes. The Ponar dredge is heavy and usually requires a boat and winch for operation.

Specialized sampling devices have been developed for sampling invertebrates on aquatic vegetation (Downing 1984). The simplest technique is the sweep net. To collect invertebrate fauna for qualitative samples, a researcher merely sweeps a net at random through stands of vegetation for a given amount of time or a given number of sweeps. Other more quantitative devices enable a worker to isolate a standard area of vegetation, clip or cut the plants, and remove the sample and associated fauna. The Wilding stovepipe sampler (APHA 1985) is a metal cylinder useful for isolating vegetation in soft sediments. A rake and net are used to remove the plants and fauna. The Macan, Minto, and McCauley samplers are more elaborate devices containing sharpened, horizontal cutting surfaces in conjunction with a sampling chamber or box.

Specific cautions must be used in interpreting data on epiphytic invertebrates (Downing 1984). Invertebrates can escape or fall from vegetation during sampling. Also, numbers may depend on macrophyte density or surface area rather than on surface area of sediment. Thus, comparisons of different vegetational densities among habitats may be biased and should be interpreted with caution. However, there may be situations in an HWS assessment, such as in littoral areas of lentic habitats, where vegetation sampling provides useful information.

Macroinvertebrates can also be semiquantitatively collected with several different varieties of standardized sampling substrates. Such substrates, which are placed into aquatic environments, can be made of "artificial" components such as tempered hardboard plates (e.g., the Hester-Dendy sampler) or of natural materials such as wire baskets containing gravel or rocks (Rosenberg and Resh 1982; Merritt and Cummins 1984; APHA 1985). Using standardized substrates to collect organisms relies on the colonization behavior of macroinvertebrates. Caution must, therefore, be used to ensure data validity; specific cautions and recommendations have been described (APHA 1985). Optimum time for colonization of substrate samplers before collection is six weeks. Care should be taken to ensure uniformity in colonization time, depth, light penetration, temperature, and current velocity (see Table 8-1 for methods) when one makes comparisons between samples obtained with standardized substrates. The benefit of these types of samplers is their comparability among sites and relative ease of use. The principal drawback is their relative selectivity in types and numbers of invertebrates collected; not all taxa are collected in the same proportions in which they occur on natural substrates. Thus, standardized samplers are considered semiquantitative techniques. If suitable reference sites are available, however, one can assume that differences among sites measured are indicative of HWS effects.

Invertebrates sampled should be isolated and preserved (APHA 1985) and identified to the desired taxonomic level. Several useful bibliographies of invertebrate keys have been published (U.S. EPA 1973; Merrit and Cummins 1984; APHA 1985). Typical endpoints include relative abundance and species (or taxon) richness. Trophic guild structure can be determined from taxonomic identifications to species (Merritt and Cummins 1984; Cummins and Wilzbach 1985). Indices of diversity, evenness, and community similarity can also be calculated.

#### 8.2.3.4 Fish

Quantifying fish population responses remains an important goal of water quality managers. Fish have been recommended for use in biomonitoring programs for at least five reasons: (1) regulators and the general public can easily understand the implications of the effects of pollution on fish; (2) fisheries have economic, recreational, and aesthetic values; (3) the identification of fishes is relatively easy (compared to that of micro- and macroinvertebrates); (4) the environmental requirements of fish are well known; and (5) fish are perceived as "integrators" of effects at lower trophic levels (Hendricks et al. 1980). However, the size, distribution, and response of freshwater fishes is sometimes difficult to quantify because variations in spatial distribution and year classes are large (Lagler 1978). Additional difficulties in the quantification of fish populations are caused by the selectivity and efficiency of the gears used (Hendricks et al. 1980). However, proper consideration of these factors can allow unbiased comparisons of different habitats, leading to a successful biomonitoring program in which fishes are useful.

Details of several techniques to quantify fish populations are described by the U.S. Environmental Protection Agency (1973), Lagler (1978), Hendricks et al. (1980), Hubert (1983) and Platts et al. (1983). Table 8-3 summarizes fish sampling methods. Two techniques proven to function well in lotic environments are electrofishing and seining. In large rivers and in lakes, most data on fish abundance and distribution are provided by electrofishing or passive netting with gill, trammel, or fyke nets (Lagler 1978).

Electrofishing is based on the principle that when a direct current is applied between two electrodes in water, fish migrate toward the anode in a galvanotaxic response; the fish are momentarily stunned and can be easily captured with a dip net. The fish recover when removed from the electric field and can be readily identified, measured, weighed, and returned to the water. Electrofishing gear ranges from small, backpack electrofishing units suitable for small, wadeable streams to large, boat-mounted rigs for large rivers and lakes. Choices of electrode design, current settings, and pulse width depend on resistivity (related to hardness, ionic strength, and turbidity) of the water and thus should be optimized (Lennon 1959). Results from electrofishing surveys are expressed as catch per unit effort (e.g., numbers or biomass collected per 15 minute interval). Proper safety precautions must be considered and applied when electrofishing; refer to Sowards (1961) for a discussion of safety considerations. Hendricks et al. (1980) recommended the judicious use of "deadman's" switches,

### Table 8-3. Sampling Methods for Fish[1]

| Method | Habitat | Effort Required[2] | |
|---|---|---|---|
| | | Persons | Time (hr) |
| electrofishing | small streams | 2 | 0.25-1 |
| | large streams, rivers, lakes | 2 | 0.25-1 |
| seining | small streams or impoundments | 2-3 | 0.50-1 |
| hoop net | streams or rivers | 2-3 | 2[2] |
| gill, trammel nets | lakes[4] | 2-3 | 2-4[3] |
| fyke net | lakes[4] | 2-3 | 2[3] |

[1] Taken from Lagler (1978); Hendricks et al. (1980); Hubert (1983); Nielsen and Johnson (1985).

[2] Time for obtaining fish sample; time for stationary netting techniques includes time spent setting and retrieving nets. It does not include time required to process sample (weighing, measuring, or taxonomic identification) which can range from 1 to 4 hours depending on taxonomic resolution and number of fish obtained.

[3] Time for hoop, gill, trammel, and fyke nets does not include 24 hours or period which net is left in water to obtain sample.

[4] Gill, trammel, and fyke nets can also be used in some cases in flowing water if properly anchored; however, debris usually makes these applications troublesome.

safety rails, felt-soled rubber boots, rubber gloves, and life jackets. Additionally, operators should be trained in electrofishing techniques, cardiopulmonary resuscitation, and electrical theory and safety.

Seines consist of long lengths of netting rigged with Styrofoam or plastic floats at the top and lead-weighted line at the bottom; a seine is usually operated by manually pulling vertical poles tied to each end of the net. Seining is most effective in streams, ponds, and nearshore areas of lakes and impoundments. In large lakes or marine waters where obstructions are few or lacking, large subsurface trawls can be pulled by boats to collect fish at different depths. Results from seining or trawling are expressed as catch per unit of effort.

Passive netting techniques are commonly used to sample fish in large rivers and lakes. Gill nets are constructed of braided or monofilament lines typically of uniform mesh size. However, to lessen the size selectivity and to increase the number of fish species collected in one net, Hubert (1983) recommended that a graded mesh size be used in gill nets. Trammel nets are modified versions of gill nets, consisting of two outer panels of large mesh netting plus an inner panel of smaller mesh. Fish pass through the large mesh and are entangled in the fine mesh netting. Gill and trammel nets are usually fished on the bottom and are anchored perpendicularly to the anticipated direction of fish movement as a vertical "fence"; as fish swim into the net, their gills become entangled. Fish caught in gill and trammel nets are often dead or injured on retrieval which may be important, depending on sampling needs and goals. These nets are usually operated overnight or for 24-hour periods. Results are expressed as number or biomass of fish per length of net per unit of time. An extensive description of gill and trammel net construction, deployment and biases is given in Hubert (1983).

Hoop nets and fyke nets are stationary nets that collect fish by entrapment. Hoop nets, consisting of mesh supported by a series of structural frames or hoops, are placed on the bottom of large streams and rivers parallel with the current. Fish are entrapped during normal, upstream movement. Most hoop nets have funnel openings to keep fish from escaping. Fyke nets are modifications of hoop nets in that they have wings or leaders that guide fish into the enclosure (Hubert 1983), and are generally used in shallower waters. Data obtained with hoop or fyke nets are recorded as number or weight of fish per net-day.

Researchers should be careful to ensure uniformity of methods (mesh sizes, sampling effort) in fish surveys. Studies of fish populations or communities often involve only relative comparisons of differences between reference and impact sites. In these instances, absolute population estimates are not needed. However, if absolute population size estimates are sought, gear selectivity must be evaluated. Lagler (1978) noted that nearly all fishing gear and sampling techniques are selective for species and sizes of fish. He described an approach to determining the sampling selectivity of gear: marked fish of different sizes are released into the population and later recaptured with the same gear; differences in the proportions of different length groups recaptured by any particular gear provide a direct measure of its selectivity. In streams (up to approximately sixth order), both upstream and downstream approaches can be blocked with seines or nets placed across the stream to prevent fish movement into or out of the sampling area. In these instances, repeated sampling, either by electrofishing or seining, yields robust estimates of fish species presence and abundance (Platts et al. 1983).

The types of analyses performed on data from the collected fish include relative abundance, species richness, and size structure. In a contaminant effects assessment program in which the fish are repeatedly sampled, population size can be estimated by using a maximum likelihood estimation technique or Zippin method after multiple-step removal-depletion sampling described by Platts et al. (1983).

One promising method for fish community assessment is the Index of Biological Integrity (IBI) (Karr 1981; Karr et al. 1986), which was developed to determine the effects of decreased habitat quality on fish communities of midwestern streams. The IBI is weighted on the basis of individual species tolerances for water quality and habitat conditions. The index is composed of 12 individual metrics divided into the fields of species composition and richness, trophic composition, abundance, and condition. Scores of each metric are classified as "best," "average," or "worst" (each class having a numerical weighting), according to information from published or other reliable ichthyological sources for streams of a given size or geographical area (Fausch et al. 1984). Typically, electrofishing or seining is used to determine the species composition and relative abundance of the fish in selected habitats. After each metric is scored, an overall score is computed ranging from 12 (poorest conditions) to 60 (best conditions).

Representative fish samples may also be taken for residue analyses for contaminant bioaccumulation. Sampling protocols for collecting fish for contaminant analyses have been published, including information on target species, collection methods, handling, preservation, shipping, chain of custody, and quality assurance (U.S. EPA 1982). Residue concentrations can serve as indicators of exposure for contaminants that bioaccumulate. Residues obtained in fish surveys can be compared to limits for consumption set by the Food and Drug Administration. However, residue information should be interpreted with caution. Many potential contaminants are ephemeral (e.g., synthetic pyrethroids), rapidly metabolized (e.g., synthetic pyrethroids and organophosphates), or biotransformed (e.g., polycyclic aromatic hydrocarbons); these characteristics sometimes make identification of parent compounds difficult (Hunn 1988). Furthermore, it is difficult to relate observed contaminant burdens to potential biological effects. Further information on measurement and interpretation of residue data is given in Chapter 7.

Some observations in fishes that have been used as biological indicators of contaminant effects are percentage of tumors (Baumann 1984; Baumann et al. 1987), vertebral anomalies (Bengtsson 1975), disease and parasites (Overstreet and Howse 1977), and fin erosion (Sherwood and Mearns 1977). Leonard and Orth (1986) urge caution in relying on these features due to several factors: mobility of fishes, statistical errors in inferences, differential species sensitivity, and subjectivity in observations. However, these observations can be useful as supportive measurements in aquatic surveys. In fact, percentage of physical anomalies in fish is one of the 12 metrics in the IBI (Karr 1981; Karr et al. 1986). Hunn (1988) provides a checklist for physical examinations of fish in field surveys, as well as other information useful for field investigations of the effects of contaminants on fishes (e.g., references and procedures for historical reconstruction of contaminant history, prediction of contaminant bioavailability, and investigations of fish kills).

### 8.2.4 Methods Integration

#### 8.2.4.1 Selection of Endpoints, Methods, and Approaches

Many criteria can be used to select endpoints for assessments of adverse effects to aquatic ecosystems (Hammons 1981; NRC 1981; Sheehan 1984). Choices of endpoints and methods depend on the needs of the survey as well as on site-specific characteristics of the HWS. Chapter 3 of this document provides information on reviewing existing information data bases, initial site assessments, formulating data objectives, and developing an assessment strategy. Hunn (1988) also provides a useful discussion of strategies for investigating the effects of contaminants on aquatic resources.

If insufficient information is available on the aquatic resources at a site, a preliminary site visit may be required to determine what aquatic resources are potentially at risk. This visit would require a basic site evaluation consisting of a physical habitat study (e.g., Platts et al. 1983; Hughes et al. 1986; Plafkin et al. 1988) and a visual biological assessment. The types of aquatic fauna present, the potential for adverse effects on biota, and the need for further biological assessment should be indicated. For instance, no aquatic survey is needed if no aquatic habitats are present. One should consider, however, that there is always an ultimate receiving body for water, even though it sometimes may be some distance from the HWS. In other situations, the HWS may be in an area that is adversely affected by other sources of chemical contamination or physical disturbance such as sedimentation. An aquatic survey may provide general information on the existing resources, but little insight into the effects contributed by the HWS itself. In these situations, toxicity tests may be more useful in determining potential risk to the aquatic environment.

If the investigator decides to conduct a preliminary aquatic field survey, the initial site evaluation should indicate appropriate control and impact assessment sites for a qualitative survey of macroinvertebrates or fish. In lotic situations, the investigator could conduct a semiquantitative, rapid bioassessment procedure on macroinvertebrates (Hilsenhoff 1988; Plafkin et al. 1988) or fish (Plafkin et al. 1988).

Initial screening tests in standing waters are more difficult, because potential gradients cannot be easily identified. A qualitative survey can be used to identify resident flora or fauna, but detection of HWS effects may be difficult. In these situations, a fish residue survey or a bioassay approach in which water, sediments, or caged organisms are used may be more useful (see Chapter 6).

Results of the preliminary site survey are used to determine later steps in the assessment sequence. If an HWS problem is indicated, additional field surveys (in conjunction with additional laboratory, on-site, or in situ bioassays) should be conducted to quantify the extent of adverse effects to the aquatic ecosystem. The advantages and limitations of using macroinvertebrates, flora, and fish have been discussed previously. It is difficult to recommend any one trophic component for study, since needs may vary in individual assessment situations (as indicated in Chapter 2 on ecological endpoints). However, there are certain instances when surveys should concentrate on a specific trophic component, such as flora (periphyton or phytoplankton), invertebrates, or fish.

Macroinvertebrates are commonly used for environmental assessments for several practical reasons: they occur in all but the most polluted of permanent aquatic habitats; they can be easily sampled by one person with little time, equipment, or experience; and they can be rather quickly identified to order or family in the field by an experienced observer.

There are several situations in which an assessment of the fish community may be needed. Fish are good bioaccumulators of contaminants and offer sufficient biomass for assessing contaminant bioavailability. This sort of assessment is important when the contaminants emission level is low. In such situations, contaminants that are not toxic may be bioaccumulated at levels that nevertheless exceed limits for human consumption. Thus, human health, recreational, and economic problems may result. Direct quantification of fish population numbers may be needed when recreational (e.g., sportfish), economic (e.g, commercial fishery), aesthetic (e.g, endangered species), or legal issues are involved.

Neither macroinvertebrates nor fish respond to nutrients or herbicides in many instances; an assessment of the primary producers is then recommended. This contingency should be evident through consideration of site history and visual observation of aquatic conditions. The choice of techniques (natural or standardized substrates) will depend on the time available for the assessment and the inherent variability of site-specific conditions.

Choosing a trophic component for surveys may also depend on the spatial scale of an HWS. When there is a defined effluent with little apparent upstream or downstream influence of other sources of contamination or habitat degradation, periphyton, macroinvertebrates, or fish could be used to detect the effects of an HWS. However, when there are numerous other point-source effluents in a stream reach, macroinvertebrates or periphyton may be more useful than fish since they are relatively immobile and respond on a spatial scale of a few meters, whereas fish may respond only on a spatial scale of a kilometer or more.

The time scale is important in intermittent or pulsed contaminant exposures. When exposures are intermittent and the time between episodes exceeds the generation time of a species, there is potential for recovery of populations or communities that could obscure the effects of an HWS. Fish communities, by virtue of their long generation time, may then be more sensitive indicators than periphyton, plankton, and macroinvertebrates.

### 8.2.4.2 Experimental Design

A sound experimental design is critical to aquatic field surveys that may be conducted during the assessment of an HWS. The design requires an understanding of the complexity of aquatic ecosystems so that confounding factors (e.g., current velocity, depth, light penetration, substrate size, organic matter, and nutrients) are controlled or accounted for in comparisons. If the sampling regime is thoughtfully planned and carefully conducted, the results enable biologists to infer causality from observed changes in numbers of individuals, species distributions, or other variables. An appropriate experimental design must be developed before a study is started; mistakes in study design cannot be "statistically corrected" after the sampling is concluded. (Chapter 4 includes information on selection of reference sites, estimation of errors, sample numbers, and appropriate data analyses.)

### 8.2.4.3 Taxonomic Resolution

Consideration must be given to the taxonomic resolution necessary to detect shifts or alterations in a biological community. Identification to species clearly requires more expertise than identifications to order, family, or genus. The degree of taxonomic resolution required will depend on the degree of environmental contamination, the intensity of effect, and the amount of time and money available for the bioassessment.

Taxonomic expertise is widely available, if sufficient time is given for identifications. Ideally, the aquatic survey is begun as early as possible to allow adequate taxonomic determinations. If sufficient time is not available, identifications to a higher taxonomic level should be made, even though some sensitivity may be lost. Costs of identification are generally nominal compared to other costs incurred in an HWS investigation. Thus, identification of taxa to genus or species should not be seen as a hindrance to field surveys.

### 8.2.5 Examples of Field Surveys

#### 8.2.5.1 Periphyton

Crossey and LaPoint (1988) used standardized granite substrates to study the effects of mine leachates on periphyton community structure and function in Prickly Pear Creek, MT. Spring Creek is a tributary contaminated with high concentrations of cadmium, copper, lead, silver, and zinc from waste piles resulting from mining, milling, and smelting in the late 1800s. Three sites on Prickly Pear Creek were studied: a control site, upstream from the confluence with Spring Creek; an impact site, immediately downstream from the confluence of Spring Creek; and a recovery site, 12 km downstream from the impact site.

Twelve granite slabs (8 X 10 cm) were placed in unshaded riffle areas of each site; sites were selected to minimize abiotic factors (current, light, temperature, and nutrients) that are important in determining rates of periphyton colonization. After 66 days of colonization, substrates were removed for measurement of structural variables (chlorophyll a, AFDM, cell number, species richness, and diversity) and functional variables (respiration, net production, and gross primary production). Also measured were dissolved metals, pH, dissolved oxygen, dissolved nutrients ($NH_3$, $NO_2^- + NO_3^-$, and $PO_4^{3-}$), photosynthetically active radiation, and current velocity.

Sites were found to be similar in all abiotic factors except for concentrations of dissolved metals, which were known historically to exceed U.S. EPA water quality standards. Periphyton community structure was found to be significantly different at the three sites. Diatom species richness and diversity were lowest in the impact zone due to the metals entering Prickly Pear Creek. Cell abundance, chlorophyll a, and AFDM of periphyton were significantly higher in the impact and recovery sites, due to the replacement of diatom species by the green alga Ulothrix sp. and the blue-green alga Chroococcus sp. Functional variables, although more variable than structural endpoints, were also altered due to the influence of metals.

### 8.2.5.2 Benthic Macroinvertebrates

Winner et al. (1980) provide an informative case study, using macroinvertebrate field surveys to quantify the effects of metals in Elam's Run and Shayler Run, two second-order streams in southwestern Ohio. Elam's Run had received fluctuating exposures of Cu, Cr, Zn, and cyanide from the effluent of a metal plating industry over an eight-year period, and Shayler Run had received a continuous dose of 120 µg/L of Cu for 30 months as part of an EPA experimental stream research project to evaluate the effects of chronic metal stress on stream fauna (Winner et al. 1975; Geckler et al. 1976). Macroinvertebrate densities in Elam's Run were determined by using an invertebrate box sampler, which sampled 0.1 $m^2$ of substrate. Macroinvertebrate densities in Shayler Run were determined with a Surber sampler, which sampled 0.09 $m^2$ of substrate. In both streams, substrate was removed from within the sampler frame and transferred to a tub of water where rocks were scrubbed with a brush. The contents of the sampler net were added to the tub; tub contents were then isolated by using a sieve for preservation and identification. Six stations at Shayler Run (0.07 km above, and 0.2, 0.8, 1.0, 1.2, and 2.6 km below the point of Cu dosing) and five stations in Elam's Run (0.4, 0.8, 1.0, 2.1, and 3.4 km downstream from the effluent) were monitored. Upstream stations were not available in Elam's Run because the areas were dry during much of the summer. Two samples of invertebrates were taken in riffle habitats at each station on each sampling date. In addition, chemical water quality variables (metals, pH, hardness, alkalinity, and conductivity) were measured.

Metal concentrations decreased downstream from point of entry in both streams. However, differences in metal concentrations were not significant between stations in Elam's Run because temporal and spatial variability were large. Macroinvertebrate densities were reduced in metal-impacted areas of both streams. Several non-insect invertebrates, including the bivalve Pisidium, the gastropod Physa, the isopod Lirceus, the flatworm Dugesia, and the crayfish Orconectes rusticus were absent or rare in Elam's Run and copper-stressed areas of Shayler Run, even though they are commonly found in other small, southwestern Ohio streams. In contrast, tubificid worms were abundant in Elam's Run.

Numbers of individuals and species richness of insects were lowest immediately below the points of metal addition, but increased with distance downstream in both streams. Mayflies occurred only in the least polluted sections of the two streams; caddisflies were numerically important in the unpolluted and intermediately polluted areas of the streams; and stoneflies were rare in all stations of both streams (a normal observation for small streams in the area). These observations support the generalization that species of mayflies, caddisflies, and stoneflies are generally sensitive indicators of the effects of metals.

The most heavily contaminated areas of the two streams were dominated by chironomids. The percentage contribution of chironomids to the invertebrate community was highly correlated with concentrations of Cu. Thus, as copper concentrations decreased downstream, the percentage contribution of chironomids to the invertebrate community decreased as well. Two species, Cricotopus bicinctus and C. infuscatus, were also numerically dominant in stations in Elam's Run containing the greatest exposure to copper. Surber (1959) also found C. bicinctus to be tolerant of metal plating wastes containing chromium, copper, and cyanide.

### 8.2.5.3 Fish

Paller et al. (1983) and Karr et al. (1985) studied the effects of chlorine and ammonia from wastewater treatment facilities on fish communities in three streams in Illinois. Copper Slough, Kaskaskia Ditch, and Saline Branch received wastewater from secondary sewage facilities, thus receiving chronic exposures of residual chlorine and ammonia. Paller et al. (1983) monitored the streams at stations above and below sewage outfalls monthly from November 1979 to June 1981 for water quality (metals, chlorophyll a, residual chlorine, ammonia, phosphate, dissolved oxygen, pH, temperature, etc.), and fish community composition. They monitored fish community composition by electrofishing a 150-m reach of stream using a three-phase, 230-V, 3000-W generator. Fish were identified by species and enumerated, and the total weight of each species was recorded. In September 1980, chlorination was discontinued at the treatment plants to determine the effect of chlorine removal on recovery of fish communities; monitoring continued monthly until the study ended.

During the study, moderately diverse fish communities were found above the outfall in all streams. Species richness ranged from 8.0 to 10.6; the fish communities were comprised of bass (Micropterus sp.), sunfish (Lepomis sp.), crappie (Pomoxis sp.), catfish (Ictalurus sp.), northern pike (Esox lucius), grass pickerel (Esox americanus), native minnows (Nocomis biguttatus, Ericymba buccata, Notropus sp., Phenacobius mirabilis, Pimephales sp., Semotilus atromaculatus, Notemigonus crysoleucas, Campostoma anomalum), suckers (Carpiodes sp., Catostomus commersoni, Erimyzon oblongus, Hypentilium nigricans, Moxostoma sp.), freshwater drum (Aplodinotus grunniens), gizzard shad (Dorosoma cepedianum), darters (Etheostoma sp.), logperch (Percina caprodes), pirate perch (Aplodinotus grunnies), topminnows (Fundulus notatus), and common carp (Cyprinus carpio). The percentage of samples composed of common carp were 36 in Copper Slough, 27 in Kaskaskia Ditch, and 65 in Saline Branch.. The IBI, calculated by Karr et al. (1985), averaged 35 to 43 in the upstream, reference areas of the three streams. Chemical analyses showed that water quality was sufficient to sustain diverse fish populations.

Samples taken downstream from the sewage outfalls during the chlorination phase of the study showed that fish populations were degraded in all streams. Species richness ranged from 3.5 in Copper Slough to 9.3 in Kaskakia Ditch. Percentages of common carp by weight increased in all streams (to 58, 75, and 71 in Kaskaskia Ditch, Copper Slough, and Saline Branch, respectively). Degradation of the fish community was further reflected in calculations of the IBI, which ranged from 21 to 31 in the three streams. Decreases in the quality of the fish community were attributed to high levels (0.5 to 1.7 mg/L) of residual chlorine in all streams; low dissolved oxygen as well as the presence of ammonia and silver were additional concerns in Saline Branch.

When effluent chlorination was stopped, the fish community recovered in downstream locations of Copper Slough and Kaskaskia Ditch; fish species richness and IBI values increased (the richness mean from 11.6 to 13.5, and the mean from 35 to 45), whereas the percentage of common carp by weight decreased to less than 18. Although residual chlorine was eliminated in Saline Branch, the fish community did not recover during the chlorine-removal portion of the study; species richness remained low (3.9) and percentage common carp by weight remained high (79) because of low dissolved oxygen (< 2.5 mg/L), and high concentrations of ammonia (0.50 mg/L, un-ionized form) and silver (.0247 mg/L).

### 8.2.6 References

American Public Health Association (APHA). 1985. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington, DC. 1268 pp.

American Society for Testing and Materials (ASTM). 1987a. Annual Book of ASTM Standards: Water and Environmental Technology, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA. 1103 pp.

American Society for Testing and Materials (ASTM). 1987b. Standard practice for sampling phytoplankton with water-sampling bottles. Pages 53-54. In: Annual Book of ASTM Standards: Water and Environmental Technology, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA.

American Society for Testing and Materials (ASTM). 1987c. Standard practice for sampling phytoplankton with pumps. Pages 45-46. In: Annual Book of ASTM Standards: Water and Environmental Technology, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA.

American Society for Testing and Materials (ASTM). 1987d. Standard practice for sampling phytoplankton with conical tow nets. Pages 42-44. In: Annual Book of ASTM Standards: Water and Environmental Technology, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA.

American Society for Testing and Materials (ASTM). 1987e. Standard practice collecting benthic macroinvertebrates with Surber and related type samplers. Pages 156-158. In: Annual Book of ASTM Standards: Water and Environmental Technology, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA.

American Society for Testing and Materials (ASTM). 1987f. Standard practice for selecting stream-net sampling devices for collecting benthic macroinvertebrates. Pages 144-155. In: Annual Book of ASTM Standards: Water and Environmental Technology, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA.

American Society for Testing and Materials (ASTM). 1987g. Standard practice for selecting grab sampling devices for collecting benthic macroinvertebrates. Pages 91-106. In: Annual Book of ASTM Standards: Water and Environmental Technology, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA.

American Society for Testing and Materials (ASTM). 1987h. Standard practice for collecting benthic macroinvertebrates with Ekman grab sampler. Pages 79-80. In:

Annual Book of ASTM Standards: Water and Environmental Technology, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA.

American Society for Testing and Materials (ASTM). 1987i. Standard practice for collecting benthic macroinvertebrates with ponar grab sampler. Pages 77-78. In: Annual Book of ASTM Standards: Water and Environmental Technology, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA.

Baumann, P.C. 1984. Cancer in wild freshwater fish populations with emphasis on the Great Lakes. J. Great Lakes Res. 10:251-253.

Baumann, P.C., W.D. Smith, and W.K. Parland. 1987. Tumor frequencies and contaminant concentrations in brown bullheads from an industrialized river and a recreational lake. Trans. Am. Fish. Soc. 116:79-86.

Bengtsson, B.E. 1975. Vertebral damage in fish induced by pollutants. Pages 48-70. In: Koeman, J.H., and J.J. Strik, eds., Sublethal Effects of Toxic Chemicals on Aquatic Animals. Elsevier Scientific, Amsterdam.

Bloesch, J., ed. 1988. Mesocosm Studies. Hydrobiologia 159:221-313. W. Junk, Publishers, Dordrecht, the Netherlands.

Courtemanch, D.L., and S.P. Davies. 1987. A coefficient of community loss to assess detrimental change in aquatic communities. Water Res. 21:217-222.

Crossey, M.J., and T.W. LaPoint. 1988. A comparison of periphyton community structural and functional responses to heavy metals. Hydrobiologia 162:109-121.

Cummins, K.W., and M.A. Wilzbach. 1985. Field procedures for analysis of functional feeding groups of stream macroinvertebrates. Contribution 1611 to Appalachian Environmental Research Laboratory, University of Maryland, Frostburg, MD. 21 pp.

DeBernardi, R. 1984. Methods for the estimation of zooplankton abundance. Pages 59-86. In: Downing, J.A., and F.H. Rigler, eds. A Manual on Methods for the Assessment of Secondary Productivity in Fresh Waters, IBP Handbook 17, Blackwell Scientific Publications, Oxford, England.

Downing, J. A. 1984. Sampling the benthos of standing waters. Pages 87-130. In: Downing, J.A., and F.H. Rigler, eds. A Manual on Methods for the Assessment of Secondary Productivity in Fresh Waters, IBP Handbook 17, Blackwell Scientific Publications, Oxford, England.

Fausch, K.D., J.R. Karr, and P.R. Yant. 1984. Regional application of an index of biotic integrity based on stream fish communities. Trans. Am. Fish. Soc. 113:39-55.

Gale, W.F., A.J. Gurzynski, and R.L. Lowe. 1979. Colonization and standing crops of epilithic algae in the Susquehanna River, Pennsylvania. J. of Phycol. 15:117-123.

Gaufin, A.R. 1958. The effects of pollution on a mid-western stream. Ohio J. Sci. 58:197-208.

Geckler, J.R., W.B. Horning, T.M. Heiheisel, Q.H. Pickering, and E.L. Robinson. 1976. Validity of laboratory tests for predicting copper toxicity in streams. EPA/600/3-76/116. U.S. Environmental Protection Agency, Duluth, MN. 192 pp.

Giesy, J.P. Jr., H.J. Kania, J.W. Bowling, R.L. Knight, S. Mashburn, and S. Clarkin. 1979. Fate and biological effects of cadmium introduced into channel microcosms. EPA/600/3-79/039, U.S. Environmental Protection Agency, Athens, GA.

Green, R.H. 1979. Sampling Design and Statistical Methods for Environmental Biologists. John Wiley and Sons, New York, NY. 257 pp.

Hamala, J.A., S.W. Duncan, and D.W. Blinn. 1981. A portable pump sampler for lotic periphyton. Hydrobiologia 80:189-191.

Hammons, A.S. 1981. Methods for Ecological Toxicology. Ann Arbor Science. Ann Arbor, MI. 310pp.

Hamilton, K., and E.P. Bergersen. 1984. Methods to Estimate Aquatic Habitat Variables. Division of Planning and Technical Services, Engineering and Research Center, U.S. Bureau of Reclamation, Denver, CO.

Hellawell, J.M. 1986. Biological Indicators of Freshwater Pollution and Environmental Management. Elsevier Applied Science Publ., London. 546 pp.

Hendricks, M.L., C.H Hocutt, and J.R. Stauffer, Jr. 1980. Monitoring of fish in lotic habitats. Pages 205-253. In: Hocutt, C.H., and J.R. Stauffer, Jr., eds. Biological Monitoring of Fish, Lexington Books, Lexington, MA.

Hersh, C.M., and W.G. Crumpton. 1987. Determination of growth rate depression of some green algae by atrazine. Bull. Environ. Contam. Toxicol. 39:1041-1048.

Hilsenhoff, W.L. 1988. Rapid field assessment of organic pollution with a family biotic index. J. North American Benthol. Soc. In Press.

Hoaglin, D.C., F. Mosteller, and J.W. Tukey. 1985. Exploring Data Tables, Trends and Shapes. John Wiley and Sons, New York, NY. 527 pp.

Hubert, W.A. 1983. Passive capture techniques. Pages 95-122. In: LA. Nielsen and D. L. Johnson, eds. Fisheries Techniques. Amer. Fish. Soc., Bethesda, MD.

Hughes, R.M., D.P. Larsen, and J.M. Omernik. 1986. Regional reference sites: A method for assessing stream potentials. Environ. Manag. 10:629-635.

Hunn, J.B. 1988. Field assessment of the effects of contaminants on fishes. Ecological Report No. 88-19, U.S. Fish and Wildlife Service, Washington, DC.

Hynes, H.B.N. 1971. Benthos of flowing water. Pages 66-80. In: Edmondson, W.T., and G.G. Winberg, eds. Secondary Productivity in Fresh Waters. IBP Handbook No. 17. Blackwell Scientific Publishers, Oxford.

Karr, J.R. 1981. Assessment of biotic integrity using fish communities. Fisheries 6:21-27.

Karr, J.R., R.C. Heidinger, and E.H. Helmer. 1985. Effects of chlorine and ammonia from wastewater treatment facilities on biotic integrity. J. Water Pollut. Control Fed. 57:912-915.

Karr, J.R., K.D. Fausch, P.L. Angermeier, P.R. Yant, and I.J. Schlosser. 1986. Assessing biological integrity in running waters: A method and its rationale. Illinois Natural History Survey Special Publication No. 5, Illinois Natural History Survey, Champaign, IL. 28 pp.

Kolkwitz, R., and M. Marsson. 1902. Grundsatz für die bilogische Beunteilung des Wassers nach seiner Flora und Fauna. Mitt. PrufAnst. WassVersorg. Abwasserbeseit. Berl. 1:33-72.

Lagler, K.F. 1978. Capture, sampling, and examination of fishes. Chap. 2. Pages 7-47. In: Fish Production in Fresh Waters, T. Bagenal, ed. IBP Handbook No. 3. Blackwell Scientific Publications, London.

Lamberti, G.A., and V.H. Resh. 1985. Distribution of benthic algae and macroinvertebrates along a thermal stream gradient. Hydrobiologia 128:13-21.

Lennon, R. E. 1959. The electrical resistivity meter in fishing investigations. Pages 1-13. In: U.S. Fish Wildl. Serv., Spec. Sci. Rep. Fish. No. 287. 13 pp.

Leonard, P.M., and D.J. Orth. 1986. Application and testing of an index of biotic integrity in small, coolwater streams. Trans. Am. Fish. Soc. 115:401-414.

Lewis, M.A., M.J. Taylor, and R.J. Larson. 1986. Structural and functional response of natural phytoplankton and periphyton communities to a cationic surfactant with considerations on environmental fate. Pages 241-268. In: Cairns, J., Jr., ed. Community Toxicity Testing. ASTM STP 920. American Society Testing and Materials, Philadelphia, PA.

Li-Cor. 1979. Radiation Measurement. Publication RMR2-1084, Li-Cor Inc., Lincoln NB.

Lind, O.T. 1979. Handbook of Common Methods in Limnology. C.V. Mosby Co., St. Louis, MO. 199 pp.

Merritt, R.W., and K.W. Cummins, eds. 1984. An Introduction to the Aquatic Insects of North America. Kendall/Hunt Publ., Dubuque, IA. 441 pp.

National Research Council (NRC). 1981. Testing for Effects of Chemicals on Ecosystems. National Academy of Sciences, National Academy Press, Washington, D.C. 103 pp.

Overstreet, R.M., and H.D. Howse. 1977. Some parasites and diseases of estuarine fishes in polluted habitats of the Mississippi. Ann. N.Y. Acad. Sci. 298:427-462.

Paine, R.T. 1969. A note on trophic complexity and community stability. Am. Nat. 103:91-93.

Paller, M.H., W.M. Lewis, R.C. Heidinger, and L.J. Wawronowicz. 1983. Effects of ammonia and chlorine on fish in streams receiving secondary discharges. J. Water Pollut. Control Fed. 55:1087-1097.

Peckarsky, B. L. 1984. Sampling the stream benthos. Pages 131-160. In: Downing, J.A., and F.H. Rigler, A Manual on Methods for the Assessment of Secondary Productivity in Fresh Waters, IBP Handbook 17, Blackwell Scientific Publications, Oxford, England.

Petersen, R.C. 1986. Population and guild analysis for interpretation of heavy metal pollution in streams. Pages 180-198. In: Cairns, J. Jr., ed. Community Toxicity Testing, ASTM STP 920. American Society for Testing and Materials, Philadelphia, PA.

Plafkin, J.L., M.T. Barbour, K.D. Porter, and S.K. Gross. 1988. Rapid bioassessment protocols for use in streams and rivers: Benthic macroinvertebrates and fish. Draft Report RTI82A, from EA Engineering, Science and Technology, Inc., to the U.S. Environmental Protection Agency, Monitoring, and Data Support Division, Washington, DC.

Platts, W.S., W.F. Megahan, and G.W. Minshall. 1983. Methods for evaluating stream, riparian, and biotic conditions. USDA Gen. Tech. Rep. INT-138, Ogden, UT. 70 pp.

Rosenberg, D.M., and V.H. Resh. 1982. The use of artificial substrates in the study of freshwater benthic macroinvertebrates. Pages 175-236. In: Cairns, J., Jr., ed. Artificial Substrates. Ann Arbor Science Publishers, Ann Arbor, MI.

Sheehan, P.J. 1984. Effects on community and ecosystem structure and dynamics. Pages 51-99. In: Sheehan, P.J., D.R. Miller, G.C. Butler, and P. Bourdeau, eds. Effects of Pollutants at the Ecosystem Level. John Wiley and Sons, Ltd., New York, NY.

Sheehan, P.J., and R.W. Winner. 1984. Comparison of gradient studies in heavy-metal polluted streams. Pages 255-271. In: Sheehan, P.J., D.R. Miller, G.C. Butler, and P. Bourdeau, eds. Effects of Pollutants at the Ecosystem Level. John Wiley and Sons, Ltd., New York, NY.

Sherwood, M.J., and A.J. Mearns. 1977. Environmental significance of fin erosion in southern California demersal fishes. Ann. N.Y. Acad. of Sci.. 298:177-189.

Siegel, S. 1956. Nonparametric Statistics for the Behavioral Sciences. McGraw-Hill Book Co., New York, NY. 312 pp.

Sowards, C.L. 1961. Safety as related to the use of chemicals and electricity in fishery management. U.S. Fish Wildl. Serv. Bur. of Sport Fish. Wildl., Branch Fish. Manage., Spearfish, SD.

Southwood, T.R.E. 1978. Ecological Methods. John Wiley and Sons, New York, NY. 524pp.

Stevenson, R.J., and R.L. Lowe. 1986. Sampling and interpretation of algal patterns for water quality assessments. Pages 118-149. In: Isom, B.G., ed. Rationale for

Sampling and Interpretation of Ecological Data. ASTM STP 894. America. Society for Testing and Materials. Philadelphia, PA.

Surber, E.W. 1959. Cricotopus bicinctus, a midge fly resistant to electroplating wastes. Trans. Am. Fish. Soc. 88:111-116.

U.S. Environmental Protection Agency. 1973. Biological field and laboratory methods for measuring the quality of surface waters and effluents. EPA/670/4-73/001, National Environmental Research Center, U.S. Environmental Protection Agency, Cincinnati, OH.

U.S. Environmental Protection Agency. 1982. Sampling protocols for collecting surface water, bed sediment, bivalves, and fish for priority pollutant analysis. Final Report. Office of Water, Regulations, and Standards, U.S. Environmental Protection Agency, Washington, DC.

U.S. Environmental Protection Agency. 1987. A compendium of superfund field operations methods. EPA/540/p-87/001. Office of Emergency and Remedial Response, U.S. Environmental Protection Agency, Washington, DC.

Vollenweider, R.A. 1974. Primary Production in Aquatic Environments. IBP Handbook No. 12. Blackwell Scientific Publications, Oxford. 225 pp.

Winner, R.W., J.S. Van Dyke, N. Caris, and M.P. Farrell. 1975. Response of the macroinvertebrate fauna to a copper gradient in an experimentally-polluted stream. Verh. Internat. Verein. Limnol. 19:2121-2127.

Winner, R.W., M.W. Boesel, and M.P. Farrell. 1980. Insect community structure as an index of heavy-metal pollution in lotic ecosystems. Can. J. Fish. Aquat. Sci. 37: 647-655.

## 8.3 VEGETATION ASSESSMENT -- Lawrence A. Kapustka

### 8.3.1 Introduction

### 8.3.1.1 Accessibility

Access to hazardous waste sites (HWSs) generally is restricted due to legal/proprietary and human health risk considerations. Restricted access imposes significant constraints on ecological assessment. However, vegetation can be analyzed in ways that overcome such access limitations.

General landscape pattern and gross structural features of vegetation can be inferred from conventional aerial photography. More sophisticated measures can be derived through remote radiometric sensing. Photosynthesis responds to environmental stress in ways that affect the spectral reflectance and fluorescence radiance emanating from a plant, and this phenomenon provides unique assessment opportunities for remote sensing. Remote sensing of vegetation affords access to restricted sites and can be used in limited cases on archived radiometric data. No other ecological community is so amenable to passive, non-intrusive assessment. Indeed, because of the dependency of other life forms on plants, quantization of plant communities by remote sensing may be the best means of acquiring preliminary estimates of impact for dependent groups (i.e., habitat structure may be useful in predicting animal use rates and exposure levels).

### 8.3.1.2 Biological Importance

Vegetation is the dominant biological component of terrestrial ecosystems, with nominally ten biomass units of plants, to four biomass units of microbial organisms, to one biomass unit of animals. Depending upon the species, soil characteristics, and environmental stresses, 40% to 85% of the plant mass resides below ground in contact with chemicals in the soil. The impact of hazardous waste on vegetation may be realized in a variety of ways and with different consequences (see Table 8-4). On the macroscale, plants are the biological source of energy as well as nutritional components for animals. Furthermore, the structure of vegetation, in concert with the varied abiotic landscape features, establishes habitat that animals rely on for protection from adverse weather and predators.

Table 8-4. Generic Negative Impacts of Hazardous Materials on Plants That Influence Vegetational Characteristics

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Primary/Direct Impacts

- quantitative suppression of plant growth
- qualitative shift in community composition and/or shift in community structure

Secondary/Indirect Impacts

- quantitative impairment of plant-microbial interactions affecting energy flow and nutrient cycling processes (decomposition, symbiotic relationships)
- altered animal use either for food or habitat

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The important features of plants for ecological assessments include the following:

- they respond to stressors found in soils through altered photosynthetic and respiratory rates;
- they harbor microbial populations in their root systems that facilitate uptake and metabolism of various organic and inorganic constituents including pollutants;
- they sequester and/or metabolize toxic substances in organs and tissues both above and below ground;
- they serve as a conduit of toxic substances into the food web; and
- they stabilize soils against wind- and water-mediated sheet erosion, thereby reducing mass transport of hazardous materials from the site.

Plants should be considered an important component of any ecological assessment of hazardous waste sites. To assess the full consequences of a contaminated site, it is crucial that analyses of the vegetation be integrated into the context of the landscape features surrounding the site. Furthermore, the plants growing in the contamination zone should receive careful consideration as candidates for toxicity testing and monitoring studies since they have already demonstrated a tolerance of the contaminants.

The vegetation growing on a site may be composed of cover crops planted specifically to stabilize soil surfaces, naturally occurring vegetation (including native and naturalized species), or some mixture of natural and planted species. As the degree of "naturalness" increases, so does the ecological complexity, and thus greater levels of analytical sophistication are required to ascertain the site's ecological condition. This section outlines an approach to vegetation assessment relevant to contaminated sites. The categories include remote sensing, direct vegetation measurements, and selected functional (or process-oriented) measurements. The objectives for each level of assessment are as follows:

Remote Sensing

- To gain current and historical information on land use and to establish generalized perspectives of landscape interactions.
- To define generalized vegetation patterns (especially gross structural attributes) suitable for habitat classification.
- To aid in defining the boundaries of impact (in some situations, especially where plants exhibit stress responses to contaminants).

Direct Vegetation Sampling

- To verify patterns discerned from remote sensing.
- To provide community composition data (i.e., species identity and dominance/density values).

Functional Processes

- To evaluate direct impacts on vegetation.
- To identify probable secondary impacts that may affect animal populations or other ecosystem processes.

Many excellent papers, texts, and manuals contain detailed descriptions of methods for vegetation sampling and analysis (e.g., Greig-Smith 1983). Often, the conditions of a hazardous waste site preclude extensive reliance on the direct techniques of vegetation sampling. The guiding principles for suggesting the measurements described in this section were couched in the following questions:

- Does the measurement provide information that allows one to document or infer ecological impact?
- Can the measurement data be obtained rapidly (i.e., minimizing on site effort and exposure time of workers) while adhering to high standards for accuracy and precision?
- Has the utility of the measurement for ecological assessment been demonstrated?

The following sections discuss vegetation assessment methods. Each of the methods discussed should be considered a Class I test.

### 8.3.2 Remote Sensing Methods

Remote sensing may be used advantageously in a number of ways to assess vegetation of hazardous waste sites. Primary sources of radiometric data are the Landsat Multi Spectral Scanner (MSS), the Thematic Mapper (TM), and the French Systeme Probatoire d'Observation de la Terre (SPOT) data banks. Resolution is the major limitation of these satellite imaging systems. Pixel resolution limits for the three types are: MSS, 80 m; TM, 30 m; and SPOT, 20 m. For improved resolution, the satellite images may be supplemented with fixed-wing aircraft flights utilizing comparable sensing equipment. The flights may also employ infrared and conventional photography. Coordinated work at individual sites for verification ("ground truthing") or for additional resolution can be performed from "cherry picker" booms with field model sensors. This tiered approach provides the following advantages:

- relatively unlimited accessibility;
- safe, non-intrusive assessment and monitoring; and
- through archived data (MSS since 1972; TM since 1982; SPOT since 1984; global coverage each 18 days), the opportunity to assess large-scale seasonal and annual vegetational patterns.

Radiometric data have been used effectively (Duinker and Nilsson 1988; Hardisky et al. 1986; Mohler et al. 1986; Rock et al. 1986; Roller and Colwell 1986; Waring et al. 1986) to accomplish the following objectives:

- to map vegetational boundaries (detecting shifts in dominant canopy species within a given forest type),
- to estimate net photosynthesis and net primary production,
- to estimate foliar nitrogen content,
- to detect drought stress,
- to detect effects from pest epidemics such as gypsy moth, and
- to assess forest decline due to air pollutants.

Conventional aerial photography should also be incorporated into the vegetation assessment. Most of the continental United States has been photographed repeatedly since 1938. Although the photographic record is incomplete and sporadic, and technical limitations (such as varied camera angle and altitude) are typically great, the photographic records contain valuable qualitative information on vegetation and land use patterns over a 50 year time span. Even subjective knowledge of generalized trends over five decades can offer important interpretive perspectives to ecological assessment.

### 8.3.3 Direct Observational Methods

The contamination characteristics of a site may require special precautionary steps to protect the personnel conducting on-site vegetational measurements. Contamination characteristics should be the primary consideration in selecting the detail of the measurement. The specific objectives of vegetation sampling should be defined early in the assessment process since the objectives dictate thoroughness and methodology options.

The first phase of direct observations should be directed toward ground truthing of the remote sensing results. This should be initiated with analysis of the off-site, uncontaminated border regions associated with the contaminated area. Clearly it is most desirable to validate the remotely sensed data with field data from the contaminated site under study. However, it may not be feasible to gain the required access to the site and the site may pose unreasonable risk to the research personnel. Even if the only validation is from adjacent border regions, the remotely sensed data will be valuable in assessing the vegetation on the affected site.

#### 8.3.3.1 Ground Truth Maps/Qualitative Assessments -- Floristics

Visiting the site is required to verify the community transitions/breaks indicated in aerial photos and to identify all prominent species. Depending on the site, multiple visits at different seasons may be needed to capture the breadth of species richness within the communities. Botanists familiar with the regional and local flora should be employed to compile the floristics checklist and to spot unusual gaps in the assemblages of species. The utility of synthetic community measures (such as the Species Diversity Indices, Indices of Similarity, etc.) are affected greatly by the degree of taxonomic discrimination associated with primary data collection.

### 8.3.3.2 Ground Truth Maps/Qualitative Assessments -- Relevee

A semiquantitative analysis of the vegetation may be sufficient to satisfy the objectives for many sites (e.g., highly disturbed and biologically isolated locales, sites that pose unacceptable risk to personnel, or sites that satisfy criteria for remote sensing analysis and only require generalized "ground-truthing"). The Relevee method (Braun-Blanquet 1932) is in effect a structured, subjective reconnaissance that uses flexible, loosely defined sampling areas (see Table 8-5) and generalized ranges of cover estimates (see Table 8-6). Additional information on growth habit (technically referred to as sociability), may be taken (see Table 8-7). Because of its subjectivity, the method may be the most cost-effective means of detecting gross differences in community organization or species assemblages associated with contamination. However, because Relevee is highly subjective and only semiquantitative, traditional parametric statistics are inappropriate to analyze the data. It is important to remember that this technique was developed to obtain information that could be used to classify similar vegetation types in discernable groups. It introduces a level of discipline in the collection of data through an otherwise subjective technique.

**Table 8-5. Estimated Minimal Area for Each Relevee Survey for Selected Vegetation Types**

| Vegetation Type | Surface Area ($M^2$) |
|---|---|
| Temperate Forest | 200 - 500 |
| Trees | 200 - 500 |
| Shrubs/herbs | 50 - 200 |
| Grassland | 50 - 100 |
| Wetlands/Meadows | 5 - 25 |

**Table 8-6. Modified Braun-Blanquet Cover Class Ranges**

| | Class Contribution to Total Cover | |
|---|---|---|
| Cover Class | Range, in % | Mean, in %[a] |
| 5 | 75 to 100 | 87.5 |
| 4 | 50 to < 75 | 62.5 |
| 3 | 25 to < 50 | 37.5 |
| 2 | 5 to < 25 | 15.0 |
| 1 | 1 to <5 | 3.0 |
| + | <1 | 0.5 |
| r | Observed but so rare as to not contribute measurably | |

[a] Note: the algebraic mid-point of the cover class range is routinely used in calculations, even though the values do not carry as many significant figures as implied.

**Table 8-7. Braun-Blanquet Plant Sociability Classes**

| Class | Criteria |
|---|---|
| 5 | occurring in large, nearly pure stands |
| 4 | occurring in large aggregates (e.g., coppice or in carpets) |
| 3 | occurring in small aggregates, clusters, or cushions |
| 2 | occurring in clumps or bunches |
| 1 | occurring singly |

In the initial design, the investigator selects a "representative" site within a particular vegetation stand. A single Relevee sample is recorded. Various stands are sampled for the purposes of classifying vegetation types. The single most important "assurance" of the quality of the data is the ability of the investigator to select the representative site within the stand based on "prior knowledge of what was typical" for the given vegetation.

For assessment of vegetation at hazardous sites, a series of Relevee samples can be collected within the affected area and from adjacent unaffected zones. These data sets can then be examined according to the traditional Bran-Blanquet classification strategy.

### 8.3.3.3 General Vegetation Sampling Strategy

Various approaches to quantitative vegetation sampling can be used for HWS assessments. Often, the details of the sampling procedure are varied to accommodate the structural and distributional features of vegetation type. Within each generalized method, the investigator has several options available (e.g., position, plotless versus defined area plots, size, shape, number, and several other factors). Greig-Smith (1983) provides a detailed theoretical treatment of vegetation sampling. Other excellent treatments of vegetation sampling, typically with fewer theoretical considerations, are Chapman (1976), Green (1979), Meyers and Shelton (1980), and Mueller-Dombois and Ellenberg (1974). Given the special constraints and considerations of hazardous waste sites, the following strategies are recommended.

8.3.3.3.1 Stratified Random Position. For each distinct vegetation type or unit (e.g., grass, shrub community, forest), divide the unit into four or more zones of approximately equal area. Distribute the sample locations (approximately equal numbers per zone) randomly within each zone.

8.3.3.3.2 Sample Size. Within each vegetation type, use either a minimum (e.g., N = 20) or an estimated sample size to achieve adequacy of sample. Adequacy of sample may be estimated according to the following equation:

$$N = [S^2 t^2]/d^2$$

where:

$N$ = sample size

$S^2$ = sample variance for density or cover

$t$ = Student's t table value for the $\alpha = 0.05$ level and the appropriate degrees of freedom (sample number used to calculate variance

$d$ = the allowable error; here for standardization purposes use 10% of the mean density or cover.

8.3.3.3.3 Plot Size, Plot Shape, and Data Collection. There is a wealth of literature devoted to determining size and shape of the sample plot and the type of data one should record for each. Trees, shrubs, and herbaceous vegetation may be considered separately. The following definitions, methods for establishing plots, and guidelines for data collection within plots are accepted widely among plant ecologists.

Trees are defined as erect, woody plants having a stem diameter $\geq$ 10 cm at 1.4 m above ground level (Diameter at Breast Height, DBH). Juveniles of tree species with lesser DBH are typically scored in the shrub category.

> **Point method**: The point-quarters method is by far the most efficient way to quantify trees. For each point, record the species, distance, and DBH of the four designated trees.
>
> **Defined Area**: Typically, a square plot 10m x 10m is established. For each tree within the plot, record the species and DBH.

Shrubs are defined as erect or prostrate woody plants (including individuals of tree species) $\leq$ 10 cm DBH.

> **Defined Area**: A plot of known area defined by a square or circular boundary (e.g., 1 $m^2$; or 2m x 2m) is established. The number of stems of each species within each plot is recorded. An estimate of canopy cover may be used as an estimator of dominance.

Herbaceous plants are all non-woody plants including bryophytes and lichens. Two different approaches to defined area sampling of herbaceous vegetation are commonly employed.

> **Cover Method:** A rectangular plot (0.1 to 1.0 $m^2$; smaller sizes used in denser vegetation) is typically segmented to aid one in estimating cover. Cover classes listed in Table 8-6 are often used. The cover value is recorded for each species present in each plot.
>
> **Harvest or Clip-plot Method:** This method is used to obtain aerial phytomass values for each species within each plot. A circular plot (0.1 to 1.0 $m^2$; smaller sizes used in denser vegetation) is established. The vegetation is severed at ground level and sorted according to species. The plant material is then dried in an oven at 70 to 80° C for 24 hours (or until constant weight is established). The material should be placed in a desiccator while it cools to room temperature (especially in humid environments) and then the weight is recorded. The raw data should be tabulated by plot and by species within each plot.

8.3.3.3.4 Collection of Stems and Roots. In addition to collecting the typical data for community descriptions, there may be reasons to collect stem and root sections or cores. Annual rings can provide direct evidence of changes in growth rates. Growth rates may be compared to known trends for a species or against rates measured for plants outside of the impacted area. Tissues may also be used to determine chemical concentrations or isotope values (discussed later) for tissues spanning the temporal ranges from pre-impact to present (or time of death of the individual).

**8.3.3.3.5 Data Summary.** Data summaries should be prepared for each discernable vegetation unit, both off site and on site. For trees, this includes the calculated estimates of density (number of individuals per hectare), basal area (the stem cross-sectional area calculated from the measures of DBH, a surrogate value for dominance), frequency (the percentage of plots having a particular species), and the importance percentage (IP, the mean of the normalized density, basal-area, and frequency values). These calculations, which are to be prepared for each species, yield average values that should be accompanied by standard error estimates (Cox 1985).

Comparable calculations are performed for the shrub and herbaceous plants. Cover estimates or phytomass values are used in place of basal area for shrubs and herbaceous plants. In the herbaceous plant sample methods, one does not acquire a measure of density.

The summary values as calculated above may be used to calculate various synthetic indices such as species diversity or coefficient of community. Extreme caution must accompany any interpretation of such values, since natural succession and stress affect the diversity of a community in non-linear patterns. Also, the indices do not provide for inclusion of variance or precision estimates. Furthermore, the effect of an HWS may be to elevate or decrease diversity. Qualitative values of harm or benefit cannot be assigned to fluxes in diversity in the absence of careful ecological analysis of the underlying features affecting a given change.

### 8.3.3.4 Symbiont Measurements

Stresses observed in plants may be indirect. The health of most plants is highly dependent upon the microbial flora residing within the root system, the rhizosphere. Associative bacteria and mycorrhizal fungi play important roles in inorganic nutrient uptake, topological complexity of root architecture, moisture stress tolerance, and "resistance" to pathological invasions. Assessments of the microbial community in terms of species richness and numbers of propagules of selected guilds offers valuable information in determining the magnitude of stress as well as the recovery potential. At present, the techniques for enumeration of the microbial populations rely on bioassays with target plants, laboratory culturing, and direct microscopic counting (Doetsch and Cook 1973). Development of sensitive detection systems for specific microbes utilizing DNA probes is underway. Within the next few years, it will be possible to test the efficacy of such advanced technologies for assessing the health of microbial systems. Until then, more traditional measures of critical microbial constituents are recommended. Following are brief descriptions of two microbial assessment techniques.

8.3.3.4.1 Vesicular Arbuscular (VA) Mycorrhizae. Select 10 species found both on- and off-site. Score the percentage colonization for at least five individuals of each species from each site. Roots should be harvested from the top 20 cm of soil. If it is impractical to harvest roots with the stem attached, take precautions to verify that the roots are from the selected plant species. The roots should be processed following the Trypan Blue staining method of Phillips and Hayman (1970) and scored for percentage colonization according to the grid-line intercept method (Giovanetti and

Mosse 1980). This procedure should be performed by a specialist trained to recognize the diagnostic features of VAM fungi (spores, arbuscules, coiled hyphae, penetration pegs, etc.). Employ 2-way ANOVA (level 1, site; level 2, species; with replication) to detect differences in mycorrhizal colonization values. See Chapter 4 for potential problems in hypothesis testing.

8.3.3.4.2 Diazotroph. Examine populations of legumes on- and off-site and score the numbers and mass of nodules. Visually check for leghemoglobin. Examine populations of actinorhizal species and compile data on nodule numbers and nodule mass. Not all areas will have legumes or actinorhizal plant species. Thus, the numbers of species and the number of specimens within species to be examined cannot be prescribed. Care should be taken to design a sampling strategy that permits valid statistical evaluation.

### 8.3.4 Process Measurement Methods

#### 8.3.4.1 Bioaccumulation of Toxic Metals

Plant samples of species found both on- and off-site should be collected and processed to determine the concentrations of nutrients and toxic metals. Representatives of various combinations of plants should be included in the samples (e.g., annuals and perennials, herbaceous and woody, fibrous root and tap root). Both aerial and root samples should be utilized. Total carbon and total nitrogen values should also be obtained to permit direct comparisons of mass and ratios of materials in the plants. Sampling design should be structured to permit statistical analyses by ANOVA. See Chapter 4 for potential problems in hypothesis testing.

### 8.3.4.2 Bioaccumulation of Organic Chemicals

Plant samples similar to those collected for metal analysis should be processed for selective analysis of xenobiotic constituents. Special precautions to minimize volatilization and metabolism of the organic chemicals must be employed. The selection of chemicals to be assayed should be guided by what is known about the types of hazardous chemicals expected to be present at the site.

### 8.3.4.3 Photosynthesis

Sophisticated methods of analyzing photosynthetic condition are available. Portable units (e.g., LICOR 6000) can be used to measure the "instantaneous" rates of net $CO_2$ uptake. There are many technical considerations that require skilled personnel to ensure reliability of the resulting data. If the proper precautions are taken, however, excellent comparative data can be obtained to assess the impact of stress imposed by hazardous materials on the photosynthetic process. The same instrument may be used to measure respiratory rates of non-photosynthetic tissues or darkened photosynthetic tissues.

There are now prototype models available of instruments that enable discrimination of the photosynthetic process into functional segments. These instruments rely upon the phenomena known as rapid fluorescence and delay fluorescence. Through a series of sensitive receptors, photomultipliers, and elaborate electronics, the instruments are able to detect the fluorescence at picosecond intervals. The rates and magnitude of fluorescent radiance allow the precise determination of the rate-limiting photosynthetic process. This approach, because it assesses the functional organization of the photosynthetic apparatus, is not subject to transient fluxes associated with the "instantaneous" measures of $CO_2$ uptake.

Another approach to assessing the photosynthetic process is isotope discrimination. The biophysical and biochemical features of leaves impose resistance to the incorporation of $CO_2$ (Farquhar et al. 1982; Hattersley 1982; O'Leary 1981). As a consequence of this resistance, plants discriminate among isotopes. This discrimination is confirmed by a comparison of the natural abundance of $^{13}C$ and $^{12}C$ to the abundance found in plants. Furthermore, the alternative photosynthetic pathways among plants exhibit differing levels of discrimination. Basically, any factor that affects the resistance of $CO_2$ influx enhances the discrimination. Thus, stressors that affect stomatal opening can be expected to alter the discrimination. Peterson and Fry (1987) provide an excellent discussion of the processes of isotope discrimination and illustrate their uses for ecosystem analyses through several case studies.

The important feature of discrimination in the context of assessing hazardous waste sites is that the process of discrimination is cumulative over extended periods of time. Thus, a low level of stress, for example 1% (a depression level not likely to be detected by any instantaneous measure), will be compounded over time. This could prove to be a very powerful tool, especially with long-lived perennial plants. To date, however, this technique has not been utilized to evaluate chemical stresses. The technology to perform the basic data collection (i.e., the measurement of isotope ratios) is well established and analyses can be performed at a cost of $30 to $100 per sample.

### 8.3.5 Recommended Assessment Approach

The following summary provides a sequential framework for assessing vegetation of hazardous waste sites. At virtually every step, decisions are made to proceed with the next level of information or to terminate the assessment. This procedure allows site conditions and objectives to guide the detail of vegetation sampling.

- Assemble site maps and aerial photos.
- Define the target zones to be measured.
- Acquire remotely sensed radiometric data.
- Develop "first cut" vegetation maps.
- Perform the required ground-truthing steps.
- Determine the general vegetation characteristics with the Relevee technique.
- Determine the importance of acquiring more detailed vegetation assessment.
- If appropriate, follow up with quantitative assessments using:
  - higher resolution remote sensing of existing vegetation (and past vegetation, as records permit)
  - quantitative, companion ground surveys
  - quantitative assessment of symbiotic associations
  - analysis of the toxic metal and xenobiotic content of plant tissues.

### 8.3.6 References

Braun-Blanquet, J. 1932. Plant Sociology: The Study of Plant Communities. McGraw Hill, New York, NY.

Chapman, S.B. 1976. Methods in Plant Ecology. John Wiley and Sons, New York, NY.

Cox, G.W. 1985. Laboratory Manual of General Ecology. W.C. Brown, Dubuque, IA.

Doetsch, R.N. and T.M. Cook. 1973. Intoduction to Bacteria and Their Ecobiology. University Park, Press, Baltimore, MD.

Duinker, P. and S. Nilsson. 1988. Proceedings: Seminar on remote sensing of forest decline attributed to air pollution. International Institute for Applied Systems Analysis, Laxenburg, Austria (EPRI EA-5715, Project 2661-19).

Farquhar, G.D., M.H. O'Leary, and J.A. Berry. 1982. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Aust. J. Plant Physiol. 9:121-137.

Giovanetti, M. and B. Mosse. 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol. 84:489-500.

Green, R.H. 1979. Sampling design and statistical methods for environmental biologists. Wiley Interscience.

Greig-Smith, P. 1983. Quantitative Plant Ecology. Third Edition. University of California Press, Berkeley, CA. 359 pp.

Hardisky, M.A., M.F. Gross, and V. Klemas. 1986. Remote sensing of coastal wetlands. Bioscience. 36:453-460.

Hattersley, P.W. 1982. Delta $^{13}C$ values of $C_4$ types in grasses. Aust. J. Plant Physiol. 9:139-154.

Meyers, W.L. and R.L. Shelton. 1980. Survey Methods for Ecosystem Management. John Wiley and Sons, New York, NY.

Mohler, R.R.J., G.L. Wells, D.R. Hallum and M.H. Trenchard. 1986. Monitoring vegetation of drought environments. Bioscience. 36:478-483.

Mueller-Dombois, D. and H. Ellenberg. 1974. Aims and Methods of Vegetation Ecology. Wiley Interscience.

O'Leary, M.H. 1981. Carbon isotope fractionation in plants. Phytochemistry. 20:553-567.

Peterson, B.J. and B. Fry. 1987. Stable isotopes in ecosystem studies. Ann. Rev. Ecol. and Syst. 18:293-320.

Phillips, J. M. and D. S. Hayman. 1970. Improved procedures for clearing and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55:158-161.

Rock, B.N., J.E. Vogelmann, D.L. Williams, A.F. Vogelmann, and T. Hoshizaki. 1986. Remote detection of forest damage. Bioscience. 36:439-445.

Roller, N.E.G. and J.E. Colwell. 1986. Coarse-resolution satellite data for ecological surveys. Bioscience. 36:468-475.

Waring, R.H., J.D. Aber, J.M. Melillo, and B. Moore, III. 1986. Precursors of change in terrestrial ecosystems. Bioscience. 36:433-438.

## 8.4 FIELD SURVEYS: TERRESTRIAL VERTEBRATES -- Karen McBee

### 8.4.1 Introduction

The purpose of this section is to review several methods for surveying populations of terrestrial vertebrates, including methods of capture or sampling, determination of demographic characteristics, and measurements of ecological diversity. Ways in which field surveys can be integrated with in situ assessments of bioaccumulation and assays of exposure and effects (see Chapter 7) are also discussed. The techniques and procedures presented in this section should be considered Class I methods.

### 8.4.2 Class I Methods

#### 8.4.2.1 Determination of Demographic Characteristics

To determine if terrestrial vertebrate populations have been adversely affected at hazardous waste sites, investigators must accurately census or estimate numbers of resident species, determine sex and age ratios, and estimate natality and mortality. Davis and Winstead (1980) review methods for estimating numbers of terrestrial vertebrate populations. They point out that accurate estimation of animal population size requires knowledge of the ecology and behavior of the species being sampled. General assumptions for any population sampling study are that mortality and recruitment during the sampling or capturing period are small and that all members of the population have an equal chance of being sampled.

Davis and Winstead (1980) classify methods for estimating animal populations into the following categories:

I. Count Animals
   A. Count all animals present in a given area - a true census.
   B. Sample counts of animals - an estimate of animals present at a given site.

II. Count Signs

   A. Count all signs in a given area - an index of a true census.

   B. Sample counts of signs - an index estimate of animals present at a given site.

A complete census of all members of a population is usually impossible, therefore sampling methods that estimate population numbers may provide the most feasible means of determining impacts on vertebrate populations at hazardous waste sites. Transect and quadrat counts are the most commonly used sampling methods. Animals can be sampled through direct observation by investigators who walk along established transects or from point-to-point within quadrats. Population sampling may also be conducted by setting trap lines along transects, or in quadrats, and recording the number and trap site of animals captured. Counts should be made for several areas within the study site or several counts of the same area should be made over a period of time. Anderson et al. (1976) review transect and quadrat sampling methods.

Estimates of animal population numbers can also be based on observation of animal "signs." The sampling design and statistical treatments are essentially the same as for direct observation or capture data. Commonly used types of sign include numbers of dens, burrows, or nests; counts of tracks, feces, songs, and calls; and counts of carcasses. Davis and Winstead (1980) question the validity and accuracy of population estimates based on counts of sign, however, and offer several cautionary comments in conducting such studies.

Many methods are available to estimate population sizes from capture studies, including sum of daily captures, cumulative sum of captures, probability of capture, catch effort, and change in some descriptive ratio (Davis and Winstead 1980). All these methods require multiple sampling periods, often over extended periods of time.

Methods of population estimation based on capture-recapture of marked individuals may provide the most accurate information on population sizes. Davis and Winstead (1980) provide detailed examples of the Lincoln Index and the Schnabel Method, (which require accumulation of capture-recapture data over an extended period of time) and the Schumacher-Eschmeyer Procedure. They discuss the advantages and shortcomings of each of these methods. Seber (1973) considers the Lincoln Index the most useful for data based on capture and recapture of marked individuals.

Knowledge of demographic parameters, such as sex and age ratios, reproductive success or natality and rearing success, and survival and mortality rates, is essential in judging the impact of polluted habitat on resident populations. Downing (1980) recommends that accurate data on population size and density plus several demographic parameters be measured at several time intervals in order to assess the status of short-lived, fluctuating species. Larger, longer-lived species may need much less intense investigation.

Information on sex ratios will indicate whether or not populations are present in sufficient numbers and proportions for normal reproductive activity. Age ratios will provide information on natality and rearing success, age-specific reproductive rates, and mortality and survival rates. Investigators should be familiar with methods to determine the sex and the age of all individuals captured in field surveys. Larson and Taber (1980) review methods for sex and age determination in birds and mammals. Sex and age ratios may be subject to bias because one sex or age group may be more easily captured than another or because seasonal differences and migration behavior may affect age and sex distributions at any given time and place (Downing 1980).

Estimates of natality and rearing success may be difficult to obtain because young are usually protected in hidden dens or nest sites and are not as active as adults, thus making them less likely to be captured. Information on natality and rearing success can be estimated from counts of nests, by recording the proportion of lactating female mammals captured, and by determining the proportion of birds that have brood patches. Examination of reproductive tracts for number and size of embryos, number of placental scars, and luteal counts in ovaries (Kirkpatrick 1980) can also provide information on fertility, fecundity, and natality.

Downing (1980) reviews methods for determining mortality and survival based on capture data. He emphasizes the shortcomings of single-sample death surveys in mortality studies and recommends that as many kinds of demographic information as possible be collected when conducting studies of the welfare of animal populations.

### 8.4.2.2 Measurements of Ecological Diversity

Measures of ecological diversity are potential tools for evaluating contaminant effects on terrestrial vertebrate communities. Species diversity data (along with information on reproduction, survivorship, and mortality of individual species) allow evaluation of current impact and predictions of potential impacts of habitat disruption on the structure and function of communities.

Community composition can be assessed by species frequency, species per unit area, spatial distribution of individuals, and numerical abundance of species (Hair 1980). Species diversity measures are among the most informative and commonly used measures of community structure (Peet 1974; Pielou 1975). Hair (1980) reviews the assumptions involved in measuring species diversity in a terrestrial community and discusses several diversity indices, including species counts, Simpson's Index, Brillouin's Formula (H), and the Shannon-Weaver Function (H').

Hair (1980) identifies two serious drawbacks to species counts. First, species counts fail to account for relative abundances of species present; and second, they are dependent on sample size. He recommends the use of "dual-concept" measures such as Simpson's Index or the Shannon-Weaver Function because they are sensitive to changes in both "species-richness" (number of different species present in a community) and "evenness" components (changes in distribution of individuals among species present). He also provides examples for calculation of several of these indices and suggests that Simpson's Index is most appropriately used when the relative dominance of a few key species is of interest.

When interpreting diversity indices it is important to remember that data from two or more sites (such as a hazardous waste site and a selected reference site) could have identical diversity index values but totally different species compositions (M'Closkey 1972). Values obtained from diversity indices are most useful when associated with other demographic parameters (Hair 1980).

### 8.4.2.3 Capturing and Sampling Techniques

Terrestrial vertebrates can be captured or sampled by hand, with mechanical devices such as traps, snares, and nets, or by use of immobilizing drugs. For some sampling techniques discussed later in this section, a visual "capture" may be sufficient.

Most mammals can be captured with a variety of commercially available traps. Leg-hold steel traps with off-set and padded jaws and conebear steel traps have been successfully used in capturing many species of carnivores and large rodents, such as beaver and nutria (Day et al. 1980). There is risk of injury or death to the animal with these traps, however, which may make their use unacceptable, especially when animals from field surveys will be used in subsequent in situ assays.

Small commercial snap-traps such as Victors and Museum Specials are used in sampling small mammal populations. Both can be successfully used to collect rats, mice, small squirrels, and shrews. But because both types are kill traps, they may cause damage to the cranium and internal organs making specimens unacceptable for use in later laboratory studies.

Box-type live traps may represent the best tool for collecting mammals in ecological assessments of hazardous waste sites. Box traps have been used successfully to capture mammals as large as deer and as small as shrews (Day et al. 1980). Several types are commercially available and many types can easily be constructed. The use of box-type live traps is advantageous because animals are less likely to be injured, they can be released for mark-recapture population studies, or they can be returned to the laboratory for use as bioaccumulators and bioindicators.

Mammals below the size of large canids can be captured with a variety of commercial live traps such as Havahart, Longworth, National, and Sherman. Sherman live traps may be the most appropriate trap for use in sampling indigenous rodent and insectivore populations at hazardous waste sites because they are inexpensive, easily transported and set, and can be thoroughly cleaned when removed from a contaminated site.

Snares have been used to capture game species, canids, and ground squirrels. Their use is reviewed by Day et al. (1980).

Conical and cylindrical pitfall traps can be used for small mammals (Nellis et al. 1974), especially burrowing insectivores such as shrews. Pitfalls may be used in association with drift fences or they may be set inserted into the ground at the edge of fallen logs or at the base of trees.

Choice of bait will depend on the species to be captured and the type of trap being used. Small box traps such as Sherman traps can be baited with chicken scratch grain or with a mixture of peanut butter and rolled oats. The peanut butter and rolled oats mixture can also be used effectively to bait snap traps. Larger box traps such as the Havahart may be baited with fruit such as apples to collect medium-sized rodents, or with chicken entrails, sardines, or canned cat food to collect carnivores.

The use of injected drugs for the capture and control of mammals has changed substantially during the past decades. Complex projectile syringes and sodium bicarbonate pressurized blow guns have made accurate delivery of drugs to the animal more certain. The number of different tranquilizing or anesthetizing drugs available for use in capturing mammals has increased greatly in the last 20 years. However, the appropriate quantity and type of drug to administer are known for very few mammals. The use of a drug in capturing animals may confound data derived from later in situ studies. Day et al. (1980) provide a thorough review of drugs, drug delivery systems, and known appropriate doses for several mammalian species.

Balgooyen (1977) reviewed capture methods for reptiles and amphibians. They included box traps similar to those used for small mammals, pitfall traps set with drift fences, pole nooses, snares, and large rubber bands. The most reliable means of capturing reptiles and amphibians is walking through the study site and turning over logs, rocks, and debris. Amphibians, water snakes, and turtles can be collected by seining, and turtles can be collected with partially submerged cone traps.

If captured animals are going to be used in population studies involving multiple recaptures or resightings, they must be marked in some easily identifiable manner. It is important that the method of marking not cause irritation or injury to the animal or hamper its normal activities. Marking methods can be permanent, semipermanent, or temporary (Day et al. 1980). Freeze-branding, tattooing, and toe-clipping are considered permanent marks. The attachment of ear tags or neck collars are considered semipermanent marks, although they may stay attached for the life of the animal. Temporary marks include dyes, fluorescent markers, and chemoluminescent tags.

Reptiles and amphibians can be marked for use in population recapture studies by freeze-branding and toe-clipping. Reptiles can be marked by scale painting or clipping.

Nets are most commonly used to capture birds, but two types have been successfully adapted for use in capturing mammals. Cannon and drop nets can be used to capture large herds of antelope and deer (Hawkins et al. 1968; Ramsey 1968). Mist nets are the best devices for capturing bats. They are most effective when placed across the entry way to roost sites or over open standing water (Tuttle 1976).

Wilbur (1967) provides several important points which must be considered when capturing birds; the relatively greater mobility of birds compared to most other terrestrial vertebrates is especially important in trap selection. Day et al. (1980) describe a number of useful box and enclosure traps which are best for waterfowl and ground foraging birds. Cannon nets can be used for capturing whole flocks of turkey, waterfowl, and many ground foraging birds. Mist nets made of very fine black or blonde nylon and ranging from 18 to 100 feet in length can be used for live capture of almost any flying bird. Wind and other weather conditions can severely hamper netting success, and capture rates will vary throughout the day. Mist netting is especially useful for birds that are difficult to lure into baited traps (Day et al. 1980).

Special methods for marking birds are reviewed by Marion and Shamis (1977) and Stonehouse (1977). Bird banding methods are standardized by the U.S. Fish and Wildlife Service.

### 8.4.3 Methods Integration

General considerations in choosing from the variety of methods described in section 8.4.2 include: type of habitat present at the hazardous waste site; size of the site; choice of species of interest; time and funding limitations; and possible integration with other types of ecological assessment information.

The size and general habitat of the hazardous waste site in question may determine the type and intensity of sampling methods and the species to be investigated. Random quadrat sampling may be most appropriate if general populational information is sought. If a single or a few key species are being investigated it may be more appropriate to seek out suitable habitat within the hazardous waste site and restrict collecting activities to those areas. Whenever possible, it is recommended that live-trapping be used as a capture method rather than kill-trapping because kill-trapping may preclude the use of animals in subsequent longer-term population assays and in in situ bioaccumulation and bioindicator tests.

Ideally, sampling should be conducted over several days and repeated seasonally. Realistically, this may not be possible at hazardous waste sites; it is important to remember, however, that inferences drawn from single sampling periods of a single day or only a few days can be suspect (Davis and Winstead 1980; Downing 1980; Hair 1980).

Minimal information to be obtained from animals captured in ecological assessment field surveys of terrestrial vertebrates should include the following:

- Taxonomic identification to species
- Sex
- Age. The accuracy of age determination may depend on whether or not the animal is to be killed.
- Reproductive condition. Again, this may depend on whether or not animals can be killed.
- Total body weight. If animals can be killed it will be beneficial to record the following information: total body weight; wet weight of particular organs such as liver, spleen and kidneys; measurement and weight of testes; presence of embryos and placental scars; and other reproductive information (see section 8.4.2.2).

If animals are to be used for in situ analysis of bioaccumulation and exposure effects it is imperative that they be handled in accordance with methods for each specific assay (see Chapter 7). If possible, animals should be returned immediately to the laboratory for processing in in situ assays. Because returning live animals to a laboratory from a field capture site is not always feasible, certain types of tissues can be collected on site or at nearby "field laboratories." Most tissues for use in bioaccumulation assays can be collected in the field, with wet organ weights recorded, and tissues transported to the laboratory stored on dry ice or in liquid nitrogen. All tissues should be collected immediately after death. Reproductive tract tissues and other tissues that may be used in histological analyses can be removed in the field and placed in 10% buffered formalin solution for transport to the laboratory.

Cytogenetic analysis of field-captured individuals requires that bone marrow be collected and processed to the point of fixation immediately after the animal's death. This process can be readily accomplished in a field laboratory (Baker et al. 1982) and fixed cell suspensions can be transported to the laboratory in liquid nitrogen for final analysis.

### 8.4.4 Examples

Following are examples from the scientific literature of field survey methods used to assess the effects of environmental alterations on terrestrial vertebrate populations. Most of these studies were not conducted at HWSs, nor do any of these studies use all the methods described in this section. Examination of these studies, however, should provide valuable information on the realistic expectations regarding the time-span required and types of data available from field surveys. These examples may suggest how field surveys of terrestrial vertebrates can be incorporated into the ecological assessment process at HWSs and reinforce the precautions previously outlined in Section 4 regarding statistical techniques applicable to HWSs. For each example, treatment plots were compared to control plots, but only differences between these nonreplicated plots could be tested statistically. Inferences beyond such comparisons would require more information.

In studies of small mammal populations of *Mus musculus*, *Peromyscus maniculatus*, and *Microtus ochrogaster* before and after spraying with the organophosphate insecticide, dimethoate, Barrett and Darnell (1967) found no evidence that the insecticide caused direct mortality in any of the mammalian species examined. They did find a shift in species composition from omnivores to herbivores. *Mus musculus* numbers declined from 68 to 37% of the composition while *Microtus ochrogaster* increased from 13 to 44% of the total composition. *Peromyscus maniculatus* numbers were not significantly altered. The alteration in species composition may have been related to the abrupt decline in number of insects rather than to a differential, direct toxicological effect.

Decline in *Microtus pennsylvanicus* population size after application of 2,4-D herbicide was attributed to changes in vegetation rather than to direct toxic effects (Spencer and Barrett 1980). A year long study of three species (*Peromyscus polionotus*, *Sigmodon hispidus*, and *Mus musculus*) in an enclosure treated with sevin, a carbamate insecticide, indicated a long-term effect on population structure changes (Pomeroy and Barrett 1975). *Sigmodon* reproduction was apparently inhibited in the sprayed area compared to a control area, while *Mus* numbers increased. *Peromyscus* did not do well in either plot. The authors suggested that the increase in numbers of *Mus* was possibly due to decreased interaction with *Sigmodon* (Pomeroy and Barrett 1975).

Examination of effects of endrin on unenclosed populations of *Microtus pennsylvanicus* and *Peromyscus maniculatus* indicated significant declines in numbers of *Microtus* immediately after application. Numbers rapidly recovered, however, and no long term toxicological effect was demonstrated. The *Peromyscus* population also was significantly reduced immediately after application and did not recover within two years, suggesting a differential population response of the two species (Morris 1970). Enclosed populations of the same two species showed a similar immediate response to endrin application. Young Microtus entering the population after spraying showed a higher survival rate than counterparts in a control population and population levels quickly grew beyond prespraying levels (Morris 1972). The herbicide Roundup had no apparent effect on survival, reproduction, or growth of Peromyscus maniculatus in a one year study (Sullivan and Sullivan 1981).

Studies of Microtus pennsylvanicus populations inhabiting the Love Canal hazardous waste site indicated that animals from the site had a population density of only about one fourth of that for reference populations. Mean life expectancies were reduced by half, and there was an apparent differential loss in old females resulting in a shift in the sex ratio over a period of a year (Rowley et al. 1983). Orthene, an organophosphate insecticide, had no apparent effect on population size, survival, or recruitment over a two year period in Microtus pennsylvanicus compared to a control population (Jett et al. 1986).

### 8.4.5 References

Anderson, D.R., J.L. Laake, B.R. Crain, and K.P. Burnham. 1976. Guidelines for line transect sampling of biological populations. Utah Coop. Wildl. Res. Unit, Logan, UT. 27 pp.

Baker, R.J., M.W. Haiduk, L.W. Robbins, A. Cadena, and B.F. Koop. 1982. Chromosomal studies of South American bats and their systematic implications. Spec. Publ. Pymatuning Lab. Ecol. 6:303-327.

Balgooyen, T.G. 1977. Collecting methods for amphibians and reptiles. U.S.D.I. Bureau of Land Manage. Tech. Note T/N. 299. 12 pp.

Barrett, G.W., and R.M. Darnell. 1967. Effects of dimethoate on small mammal populations. Amer. Midl. Nat. 77:164-175.

Davis, D.E., and R.L. Winstead. 1980. Estimating the numbers of wildlife populations. Pages 221-245. In: S.D. Schemnitz, ed. Wildlife Management Techniques Manual. Fourth Edition. The Wildlife Society, Washington, DC.

Day, G.I., S.D. Schemnitz, and R.D. Taber. 1980. Capturing and marking wild animals. Pages 61-88. In: S.D. Schemnitz, ed. Wildlife Management Techniques Manual. Fourth Edition. The Wildlife Society, Washington, DC.

Downing, R.L. 1980. Vital statistics of animal populations. Pages 247-267. In: S.D. Schemnitz, ed., Wildlife Management Techniques Manual. Fourth Edition. The Wildlife Society, Washington, DC.

Hair, J.D. 1980. Measurement of ecological diversity. Pages 269-275. In: S.D. Schemnitz, ed. Wildlife Management Techniques Manual. Fourth Edition. The Wildlife Society, Washington, DC.

Hawkins, R.D., L.D. Martoglio, and G.G. Montgomery. 1968. Cannon-netting deer. J. Wildl. Manage. 32:191-195.

Jett, D.A., J.D. Nichols, and J.E. Hines. 1986. Effect of Orthene(R) on an unconfined population of the meadow vole (Microtus pennsylvanicus). Can. J. Zool. 64:243-250.

Kirkpatrick, R.L. 1980. Physiological indices in wildlife management. Pages 99-112. In: S.D. Schemnitz, ed. Wildlife Management Techniques Manual. Fourth Edition. The Wildlife Society, Washington, DC.

Larson, J.S., and R.D. Taber. 1980. Criteria of sex and age. Pages 143-202. In: S.D. Schemnitz, ed. Wildlife Management Techniques Manual. Fourth Edition. The Wildlife Society, Washington, DC.

Marion, W.D., and J.D. Shamis. 1977. An annotated bibliography of bird marking techniques. Bird-Banding. 48:42-61.

M'Closkey, R.T. 1972. Temporal changes in populations and species diversity in a California rodent community. J. Mammal. 53:657-676.

Morris, R.D. 1970. The effects of endrin on Microtus and Peromyscus. I. Unenclosed field populations. Can. J. Zool. 50:885-896.

Morris, R.D. 1972. The effects of endrin on Microtus and Peromyscus II. Enclosed field populations. Can. J. Zool. 50:885-896.

Nellis, C.H., C.J. Terry, and R.D. Taber. 1974. A conical pitfall trap for small mammals. Northwest Sci. 48:102-104.

Peet, R.K. 1974. The measurement of species diversity. Ann. Rev. Ecol. Syst. 5:285-307.

Pielou, E. 1975. Ecological diversity. John Wiley and Sons, New York, NY. 165 pp.

Pomeroy, S.E., and G.W. Barrett. 1975. Dynamics of enclosed small mammal populations in relation to an experimental pesticide application. Amer. Midl. Nat. 93:91-106

Ramsey, C.W. 1968. Drop-net deer trap. J. Wildl. Manage. 32:187-190.

Rowley, M.H., J.J. Christian, D.K. Basu, M.A. Pawlikowski, and C.J. Paigen. 1983. Use of small mammals (voles) to assess a hazardous waste site at Love Canal, Niagara Falls, New York. Arch. Environ. Contam. Toxicol. 12:383-397.

Seber, G.A.F. 1973. The estimation of animal abundance and related parameters. Hofner Press, New York, NY. 506 pp.

Spencer, S.R., and G.W. Barrett. 1980. Meadow vole (Microtus pennsylvanicus) population response to vegetational changes resulting from 2,4-D application. Amer. Midl. Nat. 103-32-46.

Stonehouse, B., ed. 1977. Animal Marking. Univ. Park Press, Baltimore, MD. 257 pp.

Sullivan, T.P., and D.S. Sullivan. 1981. Responses of a deer mouse population to a forest herbicide application: Reproduction, growth, and survival. Can. J. Zool. 59:1148-1154.

Tuttle, M.D. 1976. Collecting techniques. Biology of bats of the New World Family Phyllostomatidae. Part I. Spec. Publ. Mus. Texas Tech Univ. 10:71-88.

Wilbur, S.R. 1967. Live-trapping North American upland game birds. U.S.D.I. Fish and Wildlife Serv. Spec. Sci. Rep., Wildl. No. 106. 37 pp.

## 8.5 TERRESTRIAL INVERTEBRATE SURVEYS -- Jerry J. Bromenshenk

### 8.5.1 Introduction

Approximately 95% of all species of animals are invertebrates. Invertebrates play crucial roles in community and ecosystem functions such as decomposition, grazing, predation, and pollination. Because invertebrates are numerous in species and individuals per species, they are relatively easy to obtain and study, and samples usually can be collected without depleting populations. Short life cycles and small size permit simple sampling techniques. In fresh-water systems, invertebrate indicator species have been utilized for many decades to assess impact to ecological communities; more recently, structural responses of aquatic invertebrate communities have become a principal form of water quality assessment (see sections 6.2 and 8.2).

Ecological endpoints measured by terrestrial invertebrate surveys range from biochemical to ecosystem-level responses. From an ecotoxicological perspective, the question is whether these measures can discriminate changes due to contaminants at the site from those due to natural variability. Although some terrestrial invertebrate survey methods have great potential utility in assessing adverse impacts at hazardous waste sites and as a benchmark for determining the success of remedial actions, none of these approaches has been universally accepted, and there are few standard methods. Nonetheless, these methods warrant consideration since the invertebrate systems may be some of the more sensitive and crucial for evaluating ecological effects associated with hazardous wastes.

### 8.5.2 Invertebrate Survey Methods

The methods described in this section complement the acute laboratory and in situ toxicity tests described in section 6.2 of this document and the bioaccumulation and biomarker tests presented in Chapter 7. Since it is often desirable to integrate field data acquisition with laboratory testing and analysis to provide a more refined and comprehensive ecological assessment, invertebrate samples can be used, in many cases, to accomplish this with a minimum of extra cost. For example, if properly preserved, specimens collected in the field provide not only information about populations and communities, but also measures of bioaccumulation, and specimens for histological, genetic, and biomarker studies. Furthermore, these investigations can be carried out retrospectively, as needed.

For the most part, terrestrial invertebrate survey methods are relatively untried at hazardous waste sites. However, data bases and established methods sometimes exist from other regulatory programs, such as the pesticide toxicity assays required for nontarget insects by the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). The Pesticide Assessment Guidelines for FIFRA contain standards for conducting acceptable tests, guidance on evaluation and reporting of data, definition of terms, and further guidance for hazard evaluations for nontarget insects (U.S. EPA 1982). Additional data bases are available from hazard assessments such as those conducted as part of the management and surveillance of nuclear and chemical wastes at Department of Energy and military facilities.

#### 8.5.2.1 Endpoints for Class I and Class II Terrestrial Invertebrate Survey Methods

Class I methods for surveys of invertebrate populations are discussed in subsection 8.5.2.2 below; Class II methods are discussed in 8.5.2.3. These methods emphasize insects and other non-microscopic invertebrates of terrestrial systems. Potential measurable ecological endpoints include, but are not limited to the following: (1) population size and estimates of related factors such as mortality, natality, and dispersal; (2) species diversity; (3) alterations of histopathological and morphological structures; (4) behavioral responses; (5) genetic alterations; (6) biomarkers such as inhibition of acetylcholinesterase; and (7) bioaccumulation endpoints. Case history and research examples can be provided for each of the above; but, for the most part, measurement protocols remain unstandardized, have not been widely applied to hazardous waste sites, or have been applied mainly in the laboratory and not in situ.

### 8.5.2.2 Class I Methods for Surveys of Invertebrate Populations

Population measurements of terrestrial invertebrates in the field probably are the most useful for assessing contaminant exposures and effects. With larger animals, populations must have small ranges (or the waste site must be very large) to avoid obscuring effects as a result of movements onto and off of the site. However, for many invertebrate populations, even the smallest waste site is "large" in comparison to the size and movements of the organisms themselves.

In addition, because of their small size, it is possible (and probably desirable) to employ bioassessment procedures that can be accomplished by bringing waste materials into the laboratory and exposing invertebrate populations under controlled conditions, or by examining populations of these organisms under controlled conditions in situ (cages), or by using free living in situ organisms. For example, Drosophila sex-linked recessive lethal and reciprocal translocations tests have long been accepted as indicators of the potential for chemicals to cause heritable gene mutations and chromosome aberrations in animal germ cells (Waterland 1979). A standardized Drosophila protocol has been used by the National Institute of Environmental Health Sciences to test over 200 hazardous chemicals. However, application of this test to evaluations of hazardous waste sites has yet to be demonstrated.

The Class I methods for invertebrate species involve sampling and contaminant testing of honey bees or harvester ants. Approaches for the use of these invertebrate species for hazardous waste site assessment are discussed in the following sections.

**8.5.2.2.1 Honey Bee Body Burdens and Bioaccumulation of Contaminants.** Honey bees are important as pollinators and as producers of honey, pollen, and wax. It is estimated that approximately one-third of the food consumed in the United States is directly or indirectly dependent on pollination by bees (McGregor 1976), a service valued at 8 to 40 billion dollars per year (Mayer 1983). In addition, they are the most studied species of invertebrate in the world. A substantial data base exists concerning bees and toxic chemicals since FIFRA requires pesticide testing for toxicity to nontarget insects, namely honey bees.

Although bees may at first appear to be an unlikely and difficult-to-manage test organism, miniature or disposable hives and the technical support readily available from state and federal agencies, bee research laboratories, and beekeepers (Bromenshenk and Preston 1986), make bee colonies an inexpensive (as low as $25 per unit) and practically self-sustaining test system. In addition, although bees may seem most appropriate for rural sites, many cities such as New York, Seattle, and San Francisco allow beekeeping within city limits and have many urban beekeepers.

The honey bee colony presents an opportunity to conduct multi-dimensional testing (from the biochemical to the population level of organization) and to make inferences to the community and ecosystem level through the pollination syndrome. Once a colony is placed on site, the unit can be easily sampled and observed to monitor exposures via bioaccumulation, as well as to determine lethal effects such as mortality, sublethal effects such as inhibition of acetylcholinesterase by organophophates, and behavioral effects such as alterations in foraging and flight activity. In addition, toxicity testing can be conducted in the laboratory. Thus, the sample unit can yield a wide array of information.

In small and large scale investigations conducted in Europe and the United States (Wallwork-Barber et al. 1982; Bromenshenk 1988) contaminant residues in or on bees, pollen, honey, wax, and propolis have been used to evaluate the dispersion of toxics. Bees are multi-media samplers, and body burdens have been shown to correlate well with levels in environmental media (Bromenshenk et al. 1985, 1988a-c). Statistical techniques such as kriging have yielded two- and three-dimensional maps of pollutant distribution, including isopol confidence limits (Bromenshenk et al. 1985). Honey bees have been used to follow spatial distributions of numerous heavy metals and radionuclides on five federal reservations (Hanford, Idaho National Engineering Laboratory, Los Alamos, Oak Ridge, and Savannah River) and of heavy metals, particularly arsenic, cadmium, and lead, at five EPA Superfund sites in Montana and Washington. Although contaminants can be examined in honey, wax, pollen, or bees, the recommended sample is the bee itself, unless the primary data requirement is the potential to transfer toxics to humans via pollen or honey. In general, contaminant levels are highest in the forager bee, and these are the easiest samples to obtain. The recommended procedure, including an example of application and data presentation, is described in Bromenshenk et al. (1985).

Bees provide a means of examining a site in the context of the surrounding region, and are best suited for examinations of relatively large sites, since their flight range is 1.6 to 3 km. For small sites where air-borne contaminants are of concern, it is feasible to constrain bees to flight cages.

Colonies of bees deployed at the site may be full-size or miniature (known as nucs by beekeepers) and can be readily obtained from local beekeepers and from suppliers of bees located in most southern states and California. Information about bees is readily obtained; all U.S. states have apicultural inspectors, generally associated with state departments of agriculture or the Agricultural Soils Conservation Service. Other sources of information and assistance are the USDA ARS bee research laboratories, particularly the Carl Hayden Bee Research Laboratory, Tucson, AZ, and the Beneficial Insects Laboratory, Beltsville, MD.

Since free-flying bees aggressively sample areas of more than 1.6 km in diameter, precise location of the sampling unit (hive) is not critical. The hive(s) should be placed near the center of the area to be sampled. Uptake of most chemical contaminants by foraging bees takes less than 24 hours. However, hives should be sampled before being placed on site to establish baseline values. Colonies moved from areas of high exposure to chemical contaminants to an area of lower exposure may take several weeks to eliminate contaminants from their colonies.

Sampling time varies from 5 to 20 minutes per hive when bees are flying. Sampling on sunny days is recommended because flight activity is curtailed on windy, rainy, or overcast days.

Laboratory requirements include analytical capability for determining the chemicals of interest at ppm, ppb, and (for some organics) ppt in biological tissues. In general, sample processing and analysis methods follow standard EPA protocols for other biological specimens.

Test outputs should be expressed as parts per million in dried bee tissue for data comparability. To date, bees have been found to be effective bioaccumulators of heavy metals, other inorganic elements such as fluoride (which they bioconcentrate), radionuclides, organic pesticides, and PCB's (Anderson and Wojtas 1986; Bromenshenk et al. 1985; Wallwork-Barber et al. 1982). The extent to which they can be used to examine non-pesticide organics such as dioxin and volatile organics is unknown, although research concerning these chemicals is ongoing.

Bees are capable of detecting extremely small concentrations of biologically available contaminants, often equalling or surpassing the capability of more traditional instrumentation. As few as 25 bees have been found to be representative of pollutant concentrations in a colony, although samples of a minimum of 200 bees are recommended. In addition, samples should be taken from a minimum of two to three hives at any location. Sample integrity, including sample custody, is essential. Sample holding times are not critical for heavy metals, but should be kept as short as possible for organics (not more than six months). Laboratory quality assurance also is essential. Although no standard reference material (SRM) is currently available for bees or any other terrestrial invertebrate tissues, the National Bureau of Standards (NBS) can supply several animal tissues -- oyster tissue (SRM 1566a), bovine liver (SRM 1577a), and albacore tuna (RM 50) as well as a variety of vegetation SRMs. In addition, a sample of cryogenically fractured bee tissue is archived in the NBS specimen bank (contact Dr. Stephan A. Wise for information).

Toxicity testing of pesticides is required for honey bees. Test protocol guidelines are published in U.S. EPA (1982).

These protocols include an acute contact LD50 laboratory test, a field-based foliar residue test, as well as recommendations for field studies. Although these regulations focus on testing for purposes of pesticide registration and labels affecting the use of pesticides, the test methods may be applicable to hazardous waste site toxicity assessments, and data bases exist for several hundred chemicals.

In addition to the acute contact LD50 laboratory test for pesticide, Wildlife International suggests a topical test similar to that of Smirle et al. (1984), who developed a topical bioassay for evaluating sublethal effects of toxins. This test has not been standardized or employed using materials from hazardous waste sites, but deserves mention as a potential method for examining responses other than acute toxicity.

There are no established protocols for field assessments of toxicity, although guidelines are provided. Likely test methods that may be incorporated into data acquisition objectives include in situ toxicity assessments of adult bee mortality by classical methods such as Todd dead bee hive entrance traps (Atkins et al. 1970), estimates of colony population size along pollutant exposure gradients (Bromenshenk et al. 1988a), and brood survival (Thomas et al. 1984; Bromenshenk et al. 1985).

8.5.2.2.2 Harvester Ant Toxicity Bioassay and Body Burdens. These ants are common in all arid and semi-arid habitats of the United States. They construct conspicuous nests and represent an organism that lives in intimate contact with the soil. Ongoing work near waste sites at the Idaho National Engineering Laboratory indicates that body burdens of these ants can be used to evaluate the spatial distribution of contaminants in soils, the potential for carrying buried wastes to the surface, and leachates in ground water (Paul Blom, pers. comm.). In addition, harvester ants exposed in petri dishes containing soil amended with toxicants (Gano et al. 1985) and irradiated with cesium-137 gamma radiation (Gano 1981) were sensitive to certain chemicals and consistently ranked these chemicals in order of greatest toxicity to ants. Thus, ant body burdens and laboratory-based toxicity testing (see also section 6.3.2) are test methods that may be incorporated into ecological assessments and may be applicable to site-specific needs.

#### 8.5.2.3 Class II Methods for Surveys of Invertebrate Populations

Various direct and derived measures of community structure, such as species richness and relative abundance, indicator species, and numerical indices of taxonomic and abundance data, have long been used to study the effects of pollutants on aquatic systems. In terrestrial systems, while interactions of air pollutants with plants and insects are well documented (especially for insect pests affecting forests and, to a lesser degree, agricultural crops), direct measures of invertebrate community structure are not usually suited to short term assessment of hazardous waste sites. Although relatively standardized insect and disease survey methods are available (Heagle 1973; Hay 1977; Alstead et al. 1982), the approaches are best suited for large-scale or long-term studies, since they involve examination of temporal and spatial patterns in large data bases. Diagnostic characteristics that are employed include: (1) pattern of insect damage relative to a known source, (2) deviations from "normal" outbreak patterns, (3) appearance of insects in outbreak levels that rarely reach epidemic levels, (4) documentation of change through time relative to the source, (5) establishment of ecological or physiological basis for the relationship, and (6) correlative statistical approaches between levels of exposure and degree of infestation or damage. Only rarely will this type of information be obtainable for hazardous waste site assessment.

In addition to the air pollution-plant-insect interactions, toxic chemicals in soil or litter frequently have been shown to have adverse effects on soil- and litter-dwelling arthropods. The sampling methods are relatively well established, usually involving sampling of a unit of soil or litter or by the placement of litter bags on the site, followed by extraction of the invertebrates using Berlese/Tullgren funnels or floatation methods (Southwood 1975; French 1970, 1971). A practical problem often arises concerning the safety of personnel attempting to sample and handle potentially highly contaminated soils and litter at a hazardous waste site. In most cases, laboratory assays of soil preparations using indicator species and tests such as the Eisenia foetida (earthworm) 14-day acute toxicity bioassay (section 6.3.2) or various microbial bioassays (section 6.4.2) reduce risks to personnel and, as such, are used as surrogate estimators of population and community responses in place of direct field surveys.

With respect to other community assessment endpoints employing terrestrial invertebrates, one-time or limited field surveys of community structure and function are unlikely to be of much use. For example, there is no terrestrial counterpart to the 100-year data base that exists for aquatic invertebrate communities. For the most part, it is difficult, if not impossible, to distinguish patterns of community structure and function that may reflect pollutant-induced perturbations from those of natural variability, which generally is high. If the community has changed, it would be revealed in terms of invertebrate species that have appeared, disappeared, or changed in relative abundance. But this is impossible to address in the absence of information about the community structure before contamination of the site. At best, all that can be accomplished is a measure of the community as it exists and of changes during and after clean up. Since comprehensive assessments of invertebrate communities such as macro- or micro-arthropods are enormously labor intensive and time-consuming and require professional assistance in the design of the sampling, taxonomic identifications of specimens, and data interpretation, this type of survey does not appear to be cost-effective and generally is not recommended.

Surveys of community structure are recommended for specific purposes. These need not be comprehensive and may consist of little more than a site visit by an entomologist or invertebrate specialist and minimal sampling, using methods such as visual observations, flushing, and collecting with a sweep net or similar device. The primary purpose is to determine the appropriateness of proceeding with on-site measurements of invertebrate population assemblages. For example, the site may be conspicuously lacking in terms of species diversity and abundance or lacking species common to the region. In addition, the site may be a potential habitat for endangered or threatened species, e.g., several species of butterflies, a moth, some beetles, or a tarantula (50 CFR Chapter I: 17.1, Subpart B and 23.23, Subpart C). Occasionally, the site may pose a threat to commercially valuable insects, such as honey bees, that may be located on or near the site. If more extensive or intensive sampling is warranted, guidelines are available (Southwood 1975; French 1970, 1971). Professional assistance should be obtained for the design, conduct, and interpretation of surveys of terrestrial invertebrate communities. Data acquisition requirements are site specific, and specific methods cannot be recommended within the scope of this document.

### 8.5.3 Methods Integration

The recommended invertebrate surveys emphasize a tiered approach and combined measures of exposure and effects. A necessary first step in a site assessment is an overview of the site itself and identification of the invertebrate population assemblages present and likely to be affected. This phase must also consider the ecological context of the site, the extent of the impacted area, and other ecological features that warrant more detailed analysis. In some cases, an invertebrate community analysis may be appropriate, but in most cases a more incisive approach is to sample or to test named species. This has the advantage of allowing the formulation and statement of clear objectives that are translatable into a practical monitoring program. The emphasis on a specific test organism may seem to be a questionable strategy, but one population generally has significance to others and, in a practical sense, we are often most concerned about a limited number of species that are ecologically or economically important, valued for aesthetic reasons, or endangered or threatened. Thus, the use of in situ or laboratory tests of acute toxicity of an organism such as an earthworm, which has an easily recognized and defined role in ecosystems, may be an appropriate choice for a site where the soils are known or thought to be highly contaminated. In addition, this type of test may prove to be an extremely valuable benchmark by which to assess the effectiveness of remedial actions.

However, a change in a measured ecological endpoint, even a statistically significant change, does not necessarily provide direct information about pollution effects. Often, survey methods provide, at best, base-line or benchmark information and some estimate of temporal and spatial variation.

A better approach is to get correlative data for the chosen measure of biological performance that correspond to changes in measured concentrations of contaminants, not only in environmental media, but in the target organisms themselves. Toxic chemicals in air, soil, or water are not necessarily hazardous unless biologically available. Questions of this nature are best addressed by organisms such as the honey bee that can be employed for assessments of exposure through bioaccumulation and for assessments of effects through tests such as acute mortality and sublethal effects. Note also that the use of an organism that can readily be utilized in the laboratory and in situ has advantages in terms of versatility and for "calibration" of field/laboratory endpoints. In addition, the ability to examine one or more endpoints at differing levels of biological organization using the same organism has cost and data interpretation benefits.

### 8.5.4 Case Studies of Invertebrate Surveys

Selected examples have been drawn from the literature to illustrate the application of invertebrate surveys in field evaluations of hazardous waste sites. None of the approaches is suitable for all hazardous waste sites, but some may have potential benefits for site-specific characterizations; nor are these examples to be taken as indicative of the only invertebrate surveys that may be employed. Other potentially useful techniques are available.

#### 8.5.4.1 Commencement Bay (Bromenshenk et al. 1985)

To show that honey bees are effective biological monitors of environmental contaminants over large areas, beekeepers of Puget Sound, WA, collected pollen and bees for chemical analysis. From these data, kriging maps of arsenic, cadmium, and fluoride were generated. Results, based on actual concentrations of contaminants in bee tissues, show that the greatest concentrations of contaminants occur close to Commencement Bay and that honey bees are effective as large-scale monitors.

In a companion study (Bromenshenk et al. 1988a), 50 mini-colonies of bees were placed along an arsenic and cadmium exposure gradient at five sites on Vashon Island in Commencement Bay. After 40 days of exposure, the mini-colonies displayed statistically significant site differences for numbers of bees and mean biomass

($P \leq .01$) and arsenic and cadmium content ($P \leq .001$). Bee populations were approximately 40% lower at the highest exposure site than at the sites of lowest exposure. Population size displayed a statistically significant ($P \leq .005$) negative correlation with arsenic content of bees.

### 8.5.4.2. Rocky Mountain Arsenal (Thomas et al. 1984)

An overall goal of the 1982 studies at the U.S. Army arsenal in Commerce City, CO (RMA) was to demonstrate that field tests using honey bees could be useful in detecting likely areas of chemical pollution. Honey bees at two waste areas, Derby Lake and Basin F, exhibited statistically higher ($P \leq .01$) brood mortality compared to hives at a control site during July and early August of 1983 (72% and 85% compared to 21%). Based on no evidence of food shortages or brood diseases, increased levels of brood mortality appeared to have resulted from contaminants brought by foraging bees to the hives.

The authors concluded that bee colonies placed near other contaminant sources would result in detection of increased brood mortality in comparison with colonies located remote from the such areas. However, personnel experienced in apiculture should conduct the tests since the occurrence of disease or natural changes in brood production patterns could be incorrectly interpreted as a response to toxic materials. These variables could be evaluated by analysis of covariance techniques.

### 8.5.5 References

Alstead, D.N., G.F. Edmonds, Jr., and L.H. Weinstein. 1982. Effects of air pollutants on insect populations. Annual Review of Entomology. 27:369-384.

Anderson, J.F. and M.A. Wojtas. 1986. Honey bees (Hymenoptera: Apidae) contaminated with pesticides and polychlorinated biphenyls. J. Econ. Entomol. 79:1200-1205.

Atkins, E.L., Jr., L.D. Anderson, and F.E. Todd. 1970. Honey bee field research aided by Todd dead bee hive entrance trap. Calif. Agric. 24:12-13.

Bromenshenk, J.J. 1988. Regional monitoring of pollutants with honey bees. Pages 156-170. In: Wise, Zeisler, Goldstein, eds. Progress in Environmental Specimen Banking. U.S. Department of Commerce, National Bureau of Standards Special Publication 740.

Bromenshenk, J.J., and E.M. Preston. 1986. Public participation in environmental monitoring: A means of attaining network capability. Pages 35-47. In: Environmental Monitoring and Assessment, Vol. 6.

Bromenshenk, J.J., S.R. Carlson, J.C. Simpson, J.M. Thomas. 1985. Pollution monitoring in puget sound with honey bees. Science. 227:632-634.

Bromenshenk, J.J, J.L. Gudatis, S.R. Carlson, and J.M. Thomas. 1988a. Sampling honey bee mini-hives for field evaluations of pollutant hazards. (Submitted to Apidologia, in review).

Bromenshenk, J.J., J.L. Gudatis, and R.C. Cronn. 1988b. Post-closure assessment of a hazardous waste site region with honey bees. (In Review).

Bromenshenk, J.J., J.L. Gudatis, and R.C. Cronn. 1988c. Heavy metal kinetics in honey bees. (In Review).

French, N.R. 1970. Field Data Collection Procedures for the Comprehensive Network 1970 Season. International Biological Program (IBP) Grassland Biome Technical Report No. 35. 37 pp.

French, N.R. 1971. Basic Field Data Collection Procedures for the Brassland Biome 1971 Season. International Biological Program (IBP) Grassland Biome Technical Report No. 85. 87 pp.

Gano, K.A., D.W. Carlile, and L.E. Rogers. 1985. A Harvester Ant Bioassay for Assessing Hazardous Chemical Waste Sites. PNL-5434, UN-11. Pacific Northwest Laboratory, Richland, WA.

Gano, K.A. 1981. Mortality of the harvester ant (Pogonomyrmex owyheei) after exposure to Cs137 gamma radiation. Environ. Entomol. 10:39-44.

Heagle, A.S. 1973. Interactions between air pollutants and plant parasites. Annual Review of Phytopathology. 11:365-388.

Hay, C.J. 1977. Bibliography on Arthropoda and Air Pollution. General Technical Report NE-34, U.S. Department of Agriculture, Forest Service.

Mayer, D.F. 1983. Man's best friend: The honeybee. In: Proceedings of the Western Apicultural Society, Sixth Annual Convention.

McGregor, S.E. 1976. Insect pollination of cultivated crop plants. Agriculture Handbook No. 46, U.S. Department of Agriculture, Agriculture Research Service. 411 pp.

Southwood, T.R.E. 1975. Ecological Methods with Particular Reference to the Study of Insect Populations. 1975 edition. Methuen and Co. LTD, London. 391 pp.

Smirle, M.J., M.L. Winston, and K.L. Woodward. 1984. Development of a sensitive bioassay for evaluating sublethal pesticide effects on the honey bee (Hymenoptera: Apidae). J. Econ. Entomol. 77:63-67.

Thomas, J.M., J.R. Skalski, L.L. Eberhardt, and M.A. Simmons. 1984. Field sampling for monitoring, migration, and defining the areal extent of chemical contamination. In: Management of Uncontrolled Hazardous Waste Site, Hazardous Material Control Research Institute, Silver Spring, MD.

U.S. Environmental Protection Agency. 1982. Pesticide assessment guidelines, subdivision L, hazard evaluation: Non-target insects. EPA/540/9-82/019. Office of Pesticides and Toxic Substances, U.S. Environmental Protection Agency, Washington, DC.

Waterland, R.L. 1979. Terrestrial ecology protocols for environmental assessment programs, workshop proceedings. EPA/600/2-79/122. Corvallis Environmental Research Laboratory, U.S. Environmental Protection Agency, Corvallis, OR.

Wallwork-Barber, M.K., R.W. Ferenbaugh, and E.S. Gladney. 1982. The use of honey bees as monitors of environmental pollution. Am. Bee J. 122:770-772.

# CHAPTER 9

# DATA INTERPRETATION

By
Donald L. Stevens, Jr., Eastern Oregon State College, La Grande, OR.

Greg Linder, NSI Technology Services Corporation,
Corvallis Environmental Research Laboratory, Corvallis, OR.

William Warren-Hicks, Kilkelly Environmental Associates, Raleigh, NC.

## 9.1 CAUSALITY

The causal link between an adverse ecological effect and a hazardous waste site (HWS) can be established by demonstrating a pattern of effects between ecological, toxicological, and chemical data. For example, the toxicity of a soil sample collected from the site can be compared to ecological survey data for terrestrial plants, invertebrates, and/or vertebrates and also compared to chemical concentrations in the soil samples. A correlation between the survey data and the toxicity and chemistry data is an indication that the ecological effects are caused by something related to the hazardous wastes. If a source of contamination can be localized, plots of toxicity and ecological data versus distance can be examined for patterns. Alternatively, isopleths of toxicity and ecological data can be prepared and evaluated. For example, a pattern of toxicity that corresponds to physical or hydrological conditions is strong indication of causality. The strength of the correspondence can be evaluated with several statistical techniques, such as regression, correlation, or nonparametric methods. If aquatic effects in flowing water are expected, toxicity at sites upstream from the HWS can be compared to toxicity at the site and at varying distances downstream. The key to establishing causality is to relate the observed differences and patterns to a reasonable physical model, and to show that the pattern is consistent across a number of endpoints. Ultimately, a preponderance of evidence is obtained demonstrating a causal link (or conversely, lack of one) between ecological, toxicological and chemical data and the HWS.

Both parametric (Snedecor and Cochran 1967) and nonparametric (Hollander and Wolfe 1973) statistical techniques can be used to assess causality. Candidate techniques include correlation, multiple regression, analysis of variance, their nonparametric equivalents, and comparisons of cumulative density functions. A competent statistician should always be consulted before an attempt is made to implement any of these methods.

To illustrate the importance of competent statistical input to the HWS assessment process, consider a hypothetical site where soil was sampled for laboratory toxicity testing, and where measures of important chemical species, measures of vegetation abundance, and other observations of biological activity could be recorded. It is reasonable to regress the LC50 values generated from the laboratory toxicity testing on several chemical species concentrations, particularly if one or more of the chemical species is known to have originated at the HWS. The presence of a significant regression of LC50 on chemical concentration would not directly indicate that the chemical species was responsible for the resultant toxicity. However, it is a direct indication that the source of the toxicity is linked to the measured chemical, and an indirect indication that the toxicity was originating from the HWS.

If the origin of toxicity can be localized, the relationship between ecological and toxicological variables and distance from the origin can be determined. This is particularly useful if there is water flowing through the site. For example, a plot of toxicity and ecological effects data against downstream distance is presented in Figure 9-1. These data show a significant relationship between toxicity and ecological effects. Similarly, recent work (Birge, et al 1989) has illustrated the role of integrated toxicological and ecological studies in assessments of complex effluents in aquatic systems,

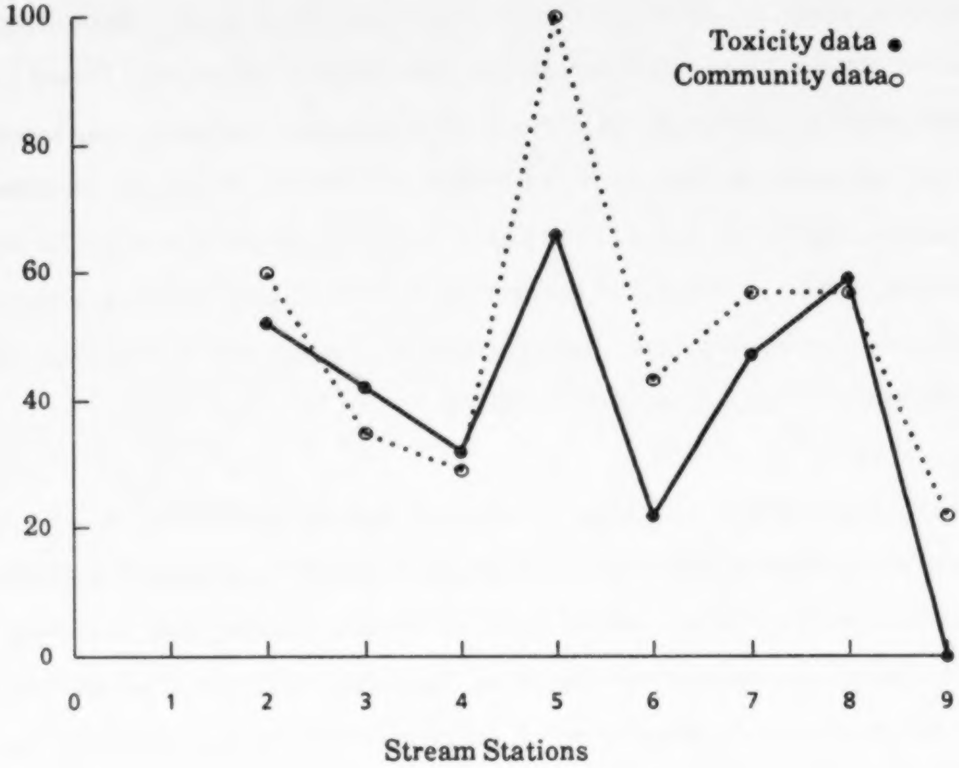

Figure 9-1. A comparison of percent toxicity and percent reduction of the taxa. (Norberg-King and Mount 1986)

## 9.2 UNCERTAINTY

Presentation of information generated from an ecological assessment of a hazardous waste site should always include an assessment of the uncertainty inherent in the data. Uncertainty is a state or condition of incomplete or unreliable knowledge. It is ubiquitous in environmental assessments and is present in most scientific endeavors.

Uncertainty also exists in all scientific projections of future conditions such as an environmental risk analysis.

Uncertainty in environmental assessment is due in part to natural variability, sampling error, measurement error, and estimation error. Sampling error uncertainty results merely from the fact that samples cannot be collected over all geographical space throughout all time. Measurement uncertainty may result from sample processing or analysis in the field or laboratory. These uncertainties may propagate themselves in the estimation of summary statistics, such as the mean or variance, or the estimation of parameters such as a coefficient in a regression equation. Uncertainty, therefore, presents a problem and a challenge for the interpretation of data generated at an HWS.

Uncertainty relates to reliability or precision, and all three terms may be used to describe the value of information. Uncertain information, uncertain statistics, and uncertain predictions are less valuable for decision making than are these same quantities when measured with less error. Therefore, estimates of uncertainty allow the decision maker to properly weigh information for which uncertainty has been assessed.

Estimating uncertainty in an ecological assessment can be a complex task. Methods for quantifying uncertainty are somewhat specific to the type of assessment, but include estimates of sample variance, confidence intervals, prediction intervals, cumulative density functions, descriptive statistics such as the inter-quartile range, and many types of graphical display techniques such as box-and-whisker plots. Whenever possible, ecological assessment data should be presented along with the appropriate estimates of uncertainty. Hypothesis testing can be significantly confounded under many types of uncertainty (see Chapter 4); therefore, exploratory techniques and graphical presentation techniques may be preferred for inferring the nature of the relationships inherent in the data.

## 9.3 ANALYSIS AND DISPLAY OF SPATIAL DATA

Much of the information collected during a field survey of an HWS will be associated with a particular spatial location, and the spatial relationship of the points will be important in interpreting the data. Maps have been used extensively to study and display spatial patterns. Many cartographic techniques are available for displaying spatially varying quantitative data. For example, if the variable being displayed is spatially continuous, it can be conceptualized as a surface in three dimensions. The surface can be displayed as contour lines, isopleths, or as perspective plots. Alternatively, if the variable is spatially discontinuous, the magnitude of an observation at a point can be represented by symbol size or color.

### 9.3.1 Point Methods

Point displays are useful for discrete spatial variables. They also give an accurate representation of the location and magnitude of observations, thus providing information not available in surface displays.

#### 9.3.1.1 Scatter Plots

Graphic techniques are an invaluable method of exploring data for relationships among several variables. Simple x-y scatter plots are one of the most effective means to detect and display relationships between two variables (Tufte 1983). Plots have an advantage over numerical techniques such as correlation or regression in that non-linear relationships and outlier data points with high leverage can become obvious. Cleveland and McGill (1984) discuss a number of techniques that can be used to enhance the information content of scatter plots. For example, a frequent problem is overplotting data, so that density of data points may be visually misjudged. Cleveland and McGill (1984) solve the overlap problem by dividing the plotting region into square subregions, counting the number of points in each subregion, and portraying the count with a "sunflower". The number of points in the subregion corresponds to the number of leaves of the sunflower: a single dot is a count of 1, a dot with a vertical line segment is count of 2, and additional line segments are added for each additional point thereafter (see Figure 9-2). Carr et al. (1986) use a similar technique, except the size of hexagonal bins is used to indicate count (see Figure 9-3).

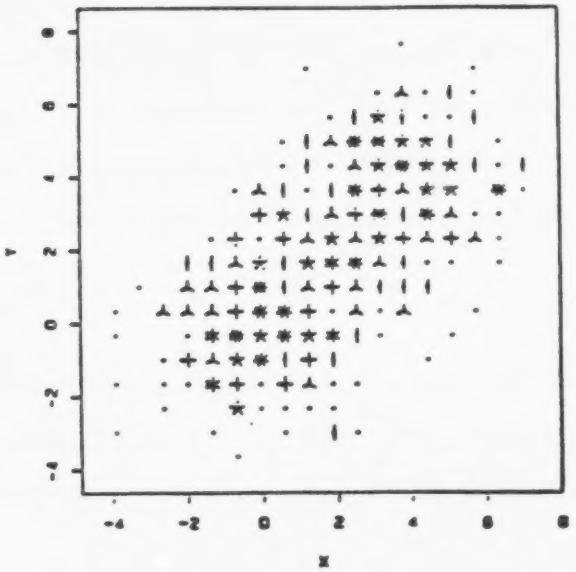

Figure 9-2. Sunflower technique for displaying clusters of data points.

Scatter plots can be used to examine multivariate relationships through the use of scatter plot matrices (Chambers et al. 1983; Cleveland McGill 1984; Carr and Nicholsen 1984). In these displays, a series of bivariate scatter plots are arranged in

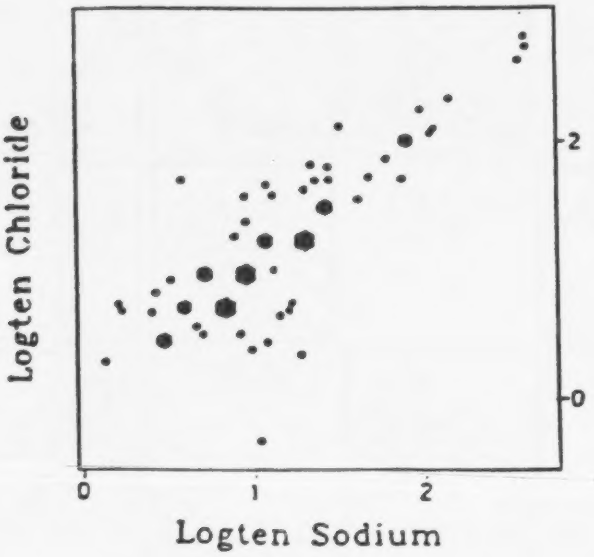

Figure 9-3. Hexagonal binning technique for displaying clusters of data points. (Carr et al. 1986)

a matrix, with all plots in the same row having the same y axis, and all plots in the same column having the same x-axis (see Figure 9-4). Often, a smooth curve is drawn through the data to aid in interpretation. The curve can be drawn by eye, or robust regression can be used to obtain a smooth curve (Cleveland 1979).

### 9.3.1.2 Glyph Plots

In the most general sense, a "glyph plot" is used to convey information by changing the appearance of a pictograph. Glyphs can be used in a coordinate-free manner to provide visual representations of multivariate data. For example, Chernoff (1973) used stylized human faces to depict associations between multivariate observations, and to identify groups with similar multivariate relationships. A "glyph plot" is much like a standard x-y scatter plot, except that information is conveyed not only by the x-y coordinates, but also by the appearance of the symbol. In a simple case, for example, the x-y axes might be map coordinates, and the size of the plotting symbol

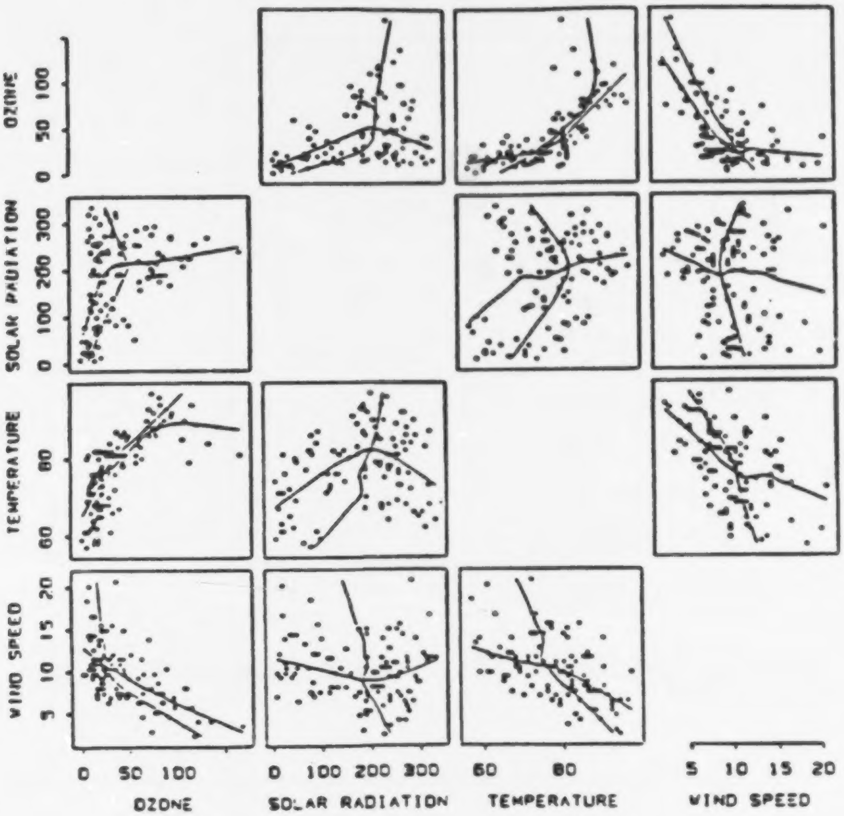

**Figure 9-4. Ozone and Meteorology data. The arrangement of the scatterplots of the four variables is called a scatterplot matrix. Each panel has a middle smoothing of y given x and of x given y, using lowess with $f = \frac{2}{3}$. The smoothings highlight the nonlinearity of the relationships among variables.**

could indicate magnitude of the observation (see Figure 9-5). Anscombe (1973) called this representation a "triple scatterplot." Additional information can be displayed by changing size or orientation of the symbol. Fienberg (1979) and Carr et al. (1986) provide overviews and discussions.

### 9.3.2 Surface Methods

In many cases, it is appropriate to think of the observations as values on a smoothly varying continuous surface, e.g., the spatial distribution of a chemical contaminant about the source of the contaminant. The surface can be represented as a three-

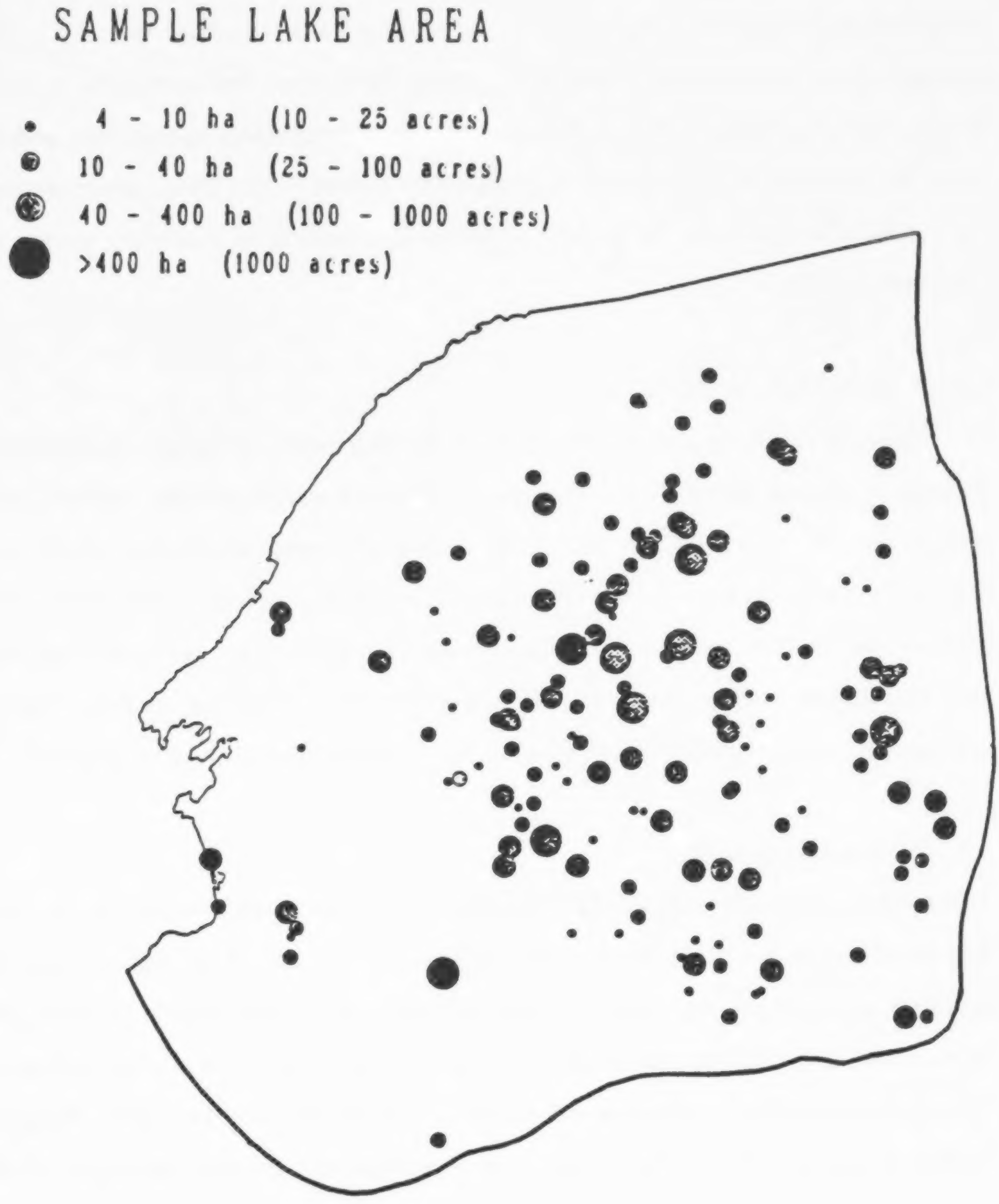
SAMPLE LAKE AREA
4 - 10 ha (10 - 25 acres)
10 - 40 ha (25 - 100 acres)
40 - 400 ha (100 - 1000 acres)
>400 ha (1000 acres)

**Figure 9-5. Example glyph plot. (Adapted from Linthurst et al. 1986)**

dimensional perspective plot or as a series of contour lines. In either case, a smooth representation requires interpolating or fitting the surface between data points. Many software packages that produce contours from irregularly spaced data points begin by interpolating the points to a regularly spaced grid. Thus, interpolation during data analysis can be avoided if systematic spacing of sampling points is achieved initially.

#### 9.3.2.1 Spatial Interpolation

Techniques in the literature that have been proposed for spatial interpolation include Thiessen polygons, polynomial interpolation, distance weighted least squares, and spatial stochastic processes. All of the commonly used methods produce an interpolated point as a weighted linear combination of observed data. The differences between the methods are in the manner in which the weights are selected. Varying assumptions are made about the underlying process that generated the data. These assumptions should be carefully checked before selecting an interpolation method.

#### 9.3.2.2 Thiessen Polygons

This method, originally published by Thiessen (1911), associates a polygon with each data point in a region, with the polygon consisting of the part of the region closer to that data point than to any other. An interpolated value at any point of interest can be obtained by assigning that point the value associated with the nearest polygon. The resulting surface is quite discontinuous, but can be the basis for a very effective display of spatial pattern. The polygons can be plotted, and the data values assigned shading intensity corresponding to magnitude (see Figure 9-6).

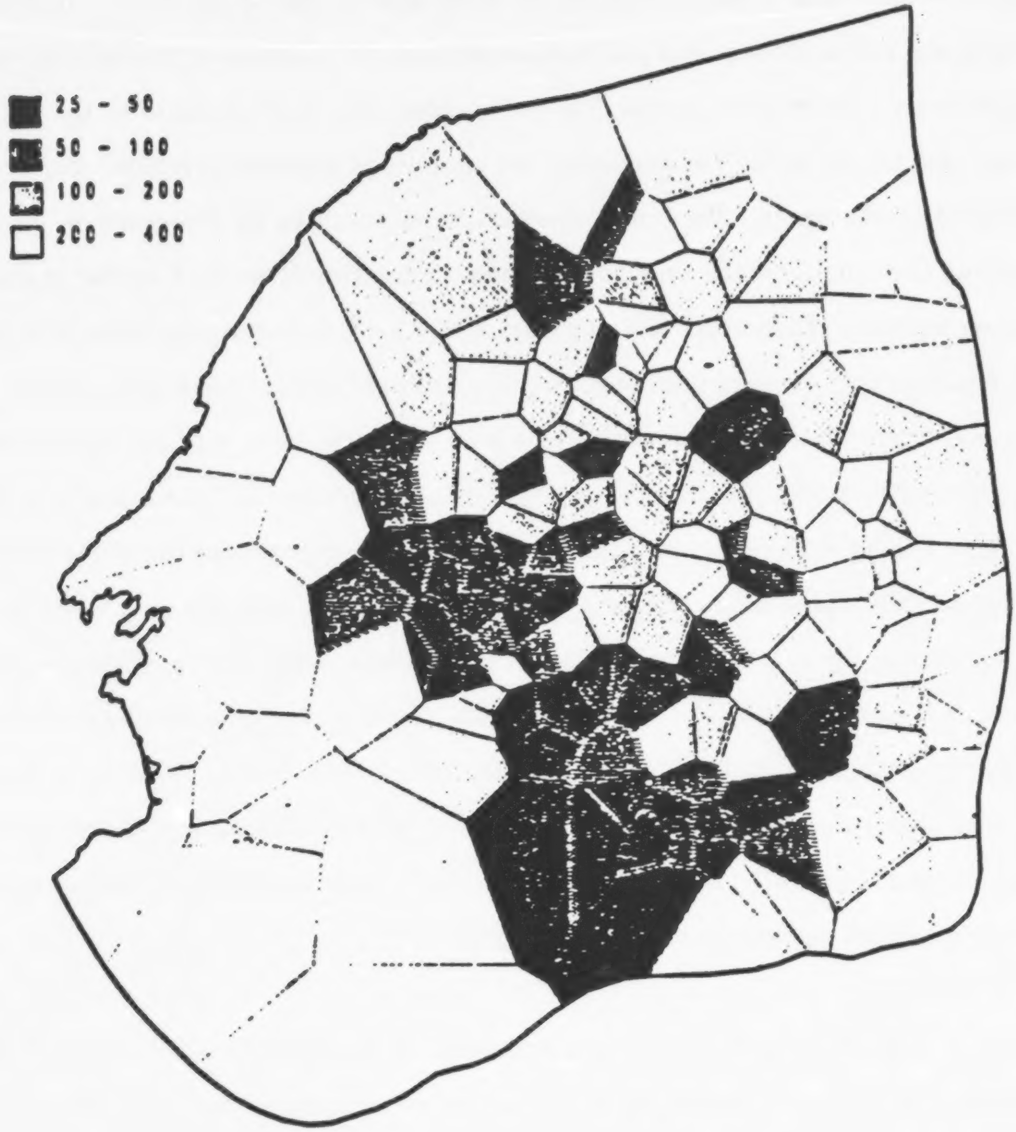
25 - 50
50 - 100
100 - 200
200 - 400

Figure 9-6. Example data depiction using Thiessen polygons. (Adapted from Linthurst et al. 1986)

### 9.3.2.3 Spatial Splines

The division of the plane region into Thiessen polygons (also called a Dirichlet tesselation) provides a starting point for some spatial spline methods. A one-dimensional spline is a series of polynomials defined over successive intervals whose endpoints are usually data points. The polynomials are "tied" together at the data points (also called "knots") by requiring the equality of adjacent polynomials when evaluated at the knots. The smoothness of the splines can be increased by also requiring the equality of the first n derivatives, where usually $n < 3$. A spatial spline requires equality of functions and derivatives along a line joining two data points. The Theissen polygons are used to construct a triangulation of the region, called a Delauney triangulation, by connecting points for which the associated polygons have a common edge. A two dimensional analog of linear interpolation fits a plane to each triangle. This produces a continuous surface, with sharp edges along the edges of the triangles. The value of the plane at a point within the triangle is a weighted combination of the values at the vertices of the triangle, where the weights are the distances from the point to the respective vertex. Several methods of using distance weighted least squares (DWLS) (McLain 1976; Akima 1978; Sibson 1980) have been proposed that interpolate over the triangles and give continuously differentiable surfaces. More generally, DWLS does not have to be restricted to interpolation over triangles, but can be used over arbitrary regions.

A related approach is to fit a bivariate polynomial to the data (Brodlie 1980). This approach leads to some smoothing if the number of monomial terms is less than the number of data points (least squares approach), or it leads to exact interpolation if the number of monomials is equal to the number of data points (LaGrange approach). A bivariate quintic polynomial is the basis of the contouring subroutine in the geographic information system ARC/INFO, marketed by ESRI (Environmental Systems Research Institute 1987a, b).

#### 9.3.2.4 Kriging

Kriging has recently become a popular technique for spatial interpolation. In this technique, the observations are considered as a realization of a spatial stochastic process with both a trend function and a noise component. The interpolated estimates are derived by minimizing the variance of the interpolation error. The estimates produced by Kriging are also weighted linear combinations of observed data. The specification of a spatial covariance structure is required in order to apply the technique, and in most applications, the covariance is assumed to be both homogeneous and isotropic. The smoothness of the resulting surface is controlled by the choice of the covariance function: the more slowly the function decreases the smoother the surface. Many Kriging applications use the variogram (a transformation of the covariance function) instead of the covariance function, but the results are equivalent. Complete discussions of Kriging can be found in Clark (1979), Journel and Huijbregts (1978) and David (1977). David (1977) also provides a thorough discussion of the basis for Kriging and discusses the practical aspects of estimating the variogram and developing a Kriging code. Davis and Culhane (1984) discuss the use of Kriging in contour applications and illustrate how to avoid a preliminary step of interpolating to a grid. Experience with Kriging as a contouring instrument has not been uniformly favorable. The contours produced sometimes cross, and behavior in regions of sparse data can be very erratic.

One advantage of Kriging over other interpolation methods is that it provides an easily available estimate of precision. The estimate can also be used to check the effects of increased sampling density. This can provide some assurance that enough data points have been taken to achieve the desired precision of the contour lines. However, the variance estimate is highly dependent on the assumed covariance function, which is one of the most difficult quantities to estimate. Large data sets are needed to provide reliable estimates, and the assumption of a homogeneous and isotropic covariance function can seldom be checked. The variance estimates should be used with caution.

Variance estimates can be obtained for any interpolation method by jackknifing, cross-validation, or bootstrapping (Efron 1981; Efron and Gong 1983). These techniques are variations on the idea of setting some data aside, and using the remaining data to predict the withheld data. Rochelle et al. (1988) provide an example of using cross-validation to estimate uncertainty in runoff contours.

## 9.4 DATA ANALYSIS AND INTERPRETATION CASE STUDIES

There are relatively few case studies that illustrate evaluations of adverse ecological effects at hazardous waste sites. The following representative examples emphasize the potential benefits gained from integrated laboratory and field assessments and reinforce the significance of gathering data on chemistry, toxicity, and ecological effects during the ecological assessment process. The realized contribution of integrated studies will vary on a site-specific basis. Ultimately, the data should be integrated for correct interpretation of the potential adverse ecological effects which may be present at an HWS.

### 9.4.1 Rocky Mountain Arsenal (Thomas et al. 1986)

In this study, laboratory toxicity test results were used in a three-phase research project with the following objectives: (1) to assess the comparative sensitivity of test organisms to known classes of chemicals; (2) to determine if the chemical components in field soil and water samples of unknown chemical composition could be inferred from laboratory studies using pure chemicals; and (3) to investigate Kriging of toxicity data as methods to define the areal extent of chemical contamination. Toxicity test results revealed that the algal assay was generally the most sensitive test for samples of pure chemicals, soil elutriates, and water from eight sites with known chemical contamination. Toxicity tests on nine samples of unknown chemical composition from the Rocky Mountain Arsenal site showed that lettuce seed germination phytoassay was the most sensitive. Preliminary evidence suggests that toxicity tests are a useful tool in identifying classes of toxic components of contaminated soil. Nearly pure formulations of insecticides and herbicides were less toxic than were their counterpart commercial formulations. This finding indicates that chemical analysis alone may fail to correctly rate the severity of possible environmental toxicity.

The case history of the Rocky Mountain Arsenal exemplifies an integrated study that incorporated laboratory-generated toxicity data into field assessments (see section 6.3). The Thomas, et al. (1986) work used toxicity test results to develop an assessment of the spatial distribution of toxicity at the site. Kriging analysis was applied to laboratory-derived toxicity test results to generate a map of the spatial distribution of toxicity (Figure 9-7). Incorporating the toxicity test results into the site assessment provided a realistic assessment of the ecological effects associated with the HWS and aided the decision making process.

### 9.4.2 Comparative Toxicity Assessment (Miller et al. 1985)

Comparative toxicological studies on algae (Selenastrum capricornutum); daphnia (Daphnia magna); earthworms (Eisenia foetida); microbes (Photobacterium fisherii), mixed sewage microorganisms and plants; wheat, "Stephans," (Triticum aestivum);

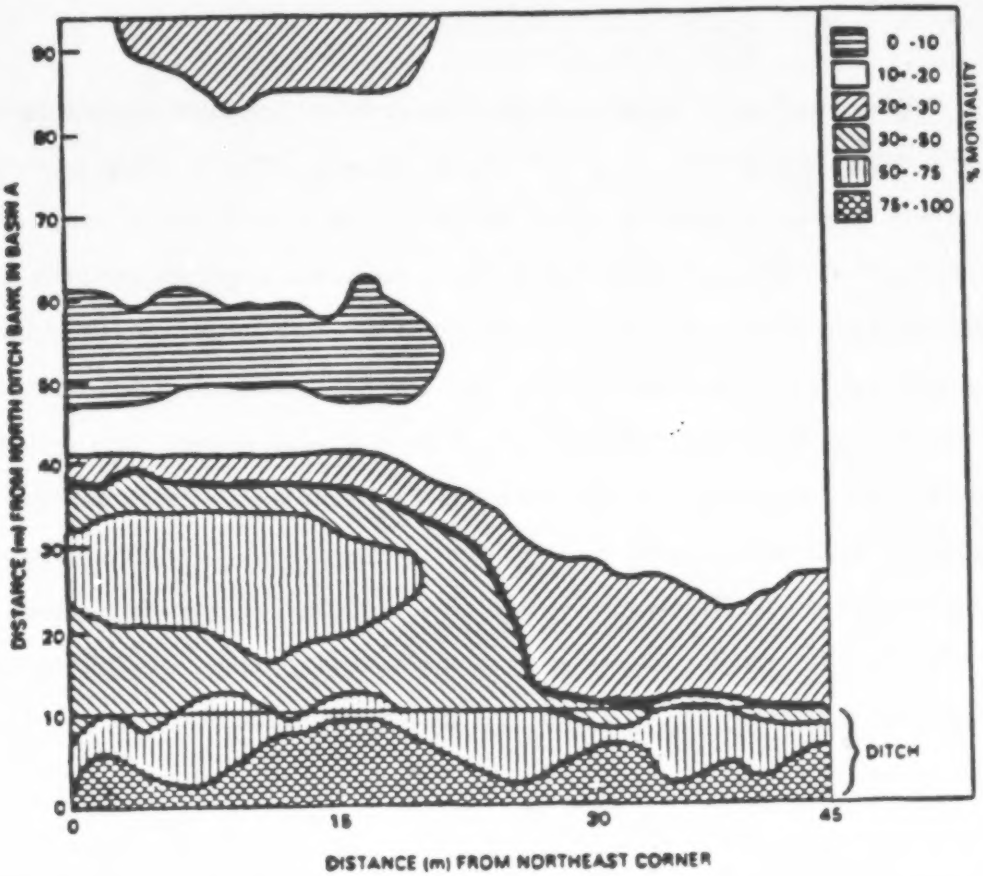
0 -10
10- -20
20- -30
30- -50
50- -75
75- -100
% MORTALITY
DITCH
DISTANCE (m) FROM NORTH DITCH BANK IN BASIN A
DISTANCE (m) FROM NORTHEAST CORNER
0
10
20
30
40
50
60
70
80
90
15
30
45

Figure 9-7. Estimated lettuce seed mortality (Based on Kriging) for the 0-15 cm soil fraction from the Rocky Mountain Arsenal. (Thomas et al. 1986)

lettuce, "butter crunch" (Lactuca sativa L.); radish, "Cherry Belle," (Raphanus sativa); red clover, "Kenland," (Trifolium partense L.); and cucumber, "Spartan Valor," (Cucumis sativa L.) were conducted on selected heavy metals, herbicides and insecticides. Algae and daphnia were found to be most sensitive to heavy metals and insecticides, followed in order of decreasing sensitivity by Microtox (Photobacterium fisherii), DO depletion rate, seed germination test, and earthworms. Higher plants were the most sensitive to 2,4-D (2,4-Dichlorophenoxy acetic acid), followed by algae, Microtox, daphnia and earthworms. Differences in toxicity of 2,4-D chemical formulations and commercial sources of insecticides were observed with algae and daphnia tests.

As part of the work, a toxicity assessment was completed for the Western Processing site in Kent, WA. Toxicity tests selected for use in this site evaluation included the earthworm test on soil as well as the algal, root elongation, and daphnid short-term tests, which were completed on surface waters and soil eluates (see Table 9-1). The battery of single-species, multi-media toxicity tests contributed significantly to the evaluation of the Western Processing site. On-site contaminant loads occurred as complex chemical mixtures rather than as single-compounds. The toxicity tests indicated that toxicity was indeed present at various locations, despite the chemical analyses of water samples that suggested that toxicity was not evident.

Table 9-1. EC50 Response in Soils (Earthworm), Soil Elutriate, and Surface Water to Chemical Contaminants in Western Processing Samples

| Test organism | | East ditch control | Pond water | Sample 005 | Sample 017 | Sample 020 |
|---|---|---|---|---|---|---|
| Algae | | 0.450 | 0.008 | 0.004 | 0.249 | NE |
| Daphnia | | 0.900 | 0.185 | 0.033 | NE[1] | NE |
| Microtox | 5 min | NE | 0.827 | 0.412 | 0.554 | NE |
| | 15 min | NE | 0.213 | 0.056 | 0.501 | NE |
| | 30 min | NE | NE | 0.056 | 0.434 | NE |
| Lettuce RE | | NE | NE | 0.614 | 0.49/1.00[2] | NE |
| Earthworms[3] | | NE | ---- | >0.50<1.00 | >1.00 | NE |

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[1] NE = No significant toxicity was observed.
[2] 49/100 = 0.49 inhibition in 1.0 soil elutriate.
[3] LC50 values = concentration at which 50% mortality occurs.

### 9.4.3 Small Mammal Assessment (Rowley et al. 1983)

In this study, voles (Microtus pennsylvanicus) were trapped in the immediate area of Love Canal near Niagra Falls, New York (I), in an area very close to Love Canal (II), and in a reference area (III) about 1 km from Love Canal. The population densities were low in I, intermediate in II, and high in III. Using ages estimated on the basis of dry lens weights, mean life expectancy from weaning was 23.6 days in I, 29.2 days in II, and 48.8 days in III. Survivorship curves had significantly steeper slopes near the canal than in the reference area. Thus, voles near the canal experienced a higher mortality rate than those in the reference area. Liver and adrenal weights in females and seminal vesicle weights in males were significantly reduced in I compared to III. A fat pool from voles in I and II contained hexachlorocyclohexane and other chlorinated hydrocarbons that were not found in voles from the reference area. These results suggest that the relatively sedentary small native mammals were useful in assessing the presence of hazardous contamination.

As in situ biomonitors, the small mammals trapped at various locations on or near the Love Canal site suggested exposure had occurred. Biological responses (e.g., altered age structure and mortality curves) suggested population level changes had occurred, and while acute toxicity was not considered in the reported work, longer-term effects related to reproductive endpoints were demonstrated in the field work completed on site or at a reference site located nearby. Supporting laboratory analyses of biological tissues (e.g., comparison of liver and adrenal weights from individuals captured on site and off site) further suggested that exposure had occurred, and reinforced the potential role of integrated laboratory and field studies as complementary features of site evaluation.

### 9.4.4 Mutagenesis Assessment (McBee et al. 1987)

In this study, examination of standard metaphase chromosome preparations was employed to evaluate the use of resident small mammals as indicators of environmental mutagenesis. Small mammals of two species, Peromyscus leucopus and Sigmodon hispidus, were trapped over a two-year period at a locality polluted with a complex mixture of petrochemical waste products, heavy metals, and PCBs (polychlorinated biphenyls) and at two uncontaminated localities. Significant differences in levels of chromosomal aberrations between animals collected at the contaminated site and the uncontaminated sites were clearly indicated. Increases in lesions per cell and aberrant cells per individual were shown for both species at the contaminated site compared to the control sites. Levels of chromosomal aberrations were not different between the two control sites, however. This study suggests that cytogenetic analysis of resident small mammals is a feasible test model for assessment of environmental mutagenesis.

As shown in Table 9-2 and Figures 9-8 and 9-9, trapped populations of small mammals presented cellular and molecular level responses (e.g., chromosomal aberrations) that were correlated with exposure to chemical constituents of complex mixtures characteristic of hazardous waste sites; acute toxicity was not addressed, nor was it apparent, in these studies. The potential longer-term biological effects suggested by the cytogenetic analyses, however, clearly indicated responses relevant to site assessments evaluating adverse ecological effects, and reinforced the importance of reference sites when correlative analyses are considered in the assessment of biological effects in the field.

Table 9-2. Chromosome Aberrations in Peromyscus leucopus from One Field Site (FS) and Two Control Sites (CS1 and CS2) as Assessed by Standard Metaphase Chromosome Preparations

| Locality | Number of individuals | Number of cells | Mean Number aberrant cells/ individual | Mean Number lesions/cell | % Cells with chromosome aberrations |
|---|---|---|---|---|---|
| CS1 | 12 | 600 | 1.42(0-4) | 0.04(0-8) | 2.83 |
| CS2 | 14 | 700 | 1.79(0-4) | 0.04(0-5) | 3.57 |
| FS1a | 12 | 600 | 5.92*t(2-10) | 0.16*t(0-15) | 11.83* |
| FS2a | 20 | 1000 | 6.15*t(2-9) | 0.15*t(0-17) | 10.06* |

* Indicates significant increases in field site values compared to the baseline value of control sites.

t Indicates significant differences by Student's t tests ($p < 0.05$). Numbers in parentheses are ranges.

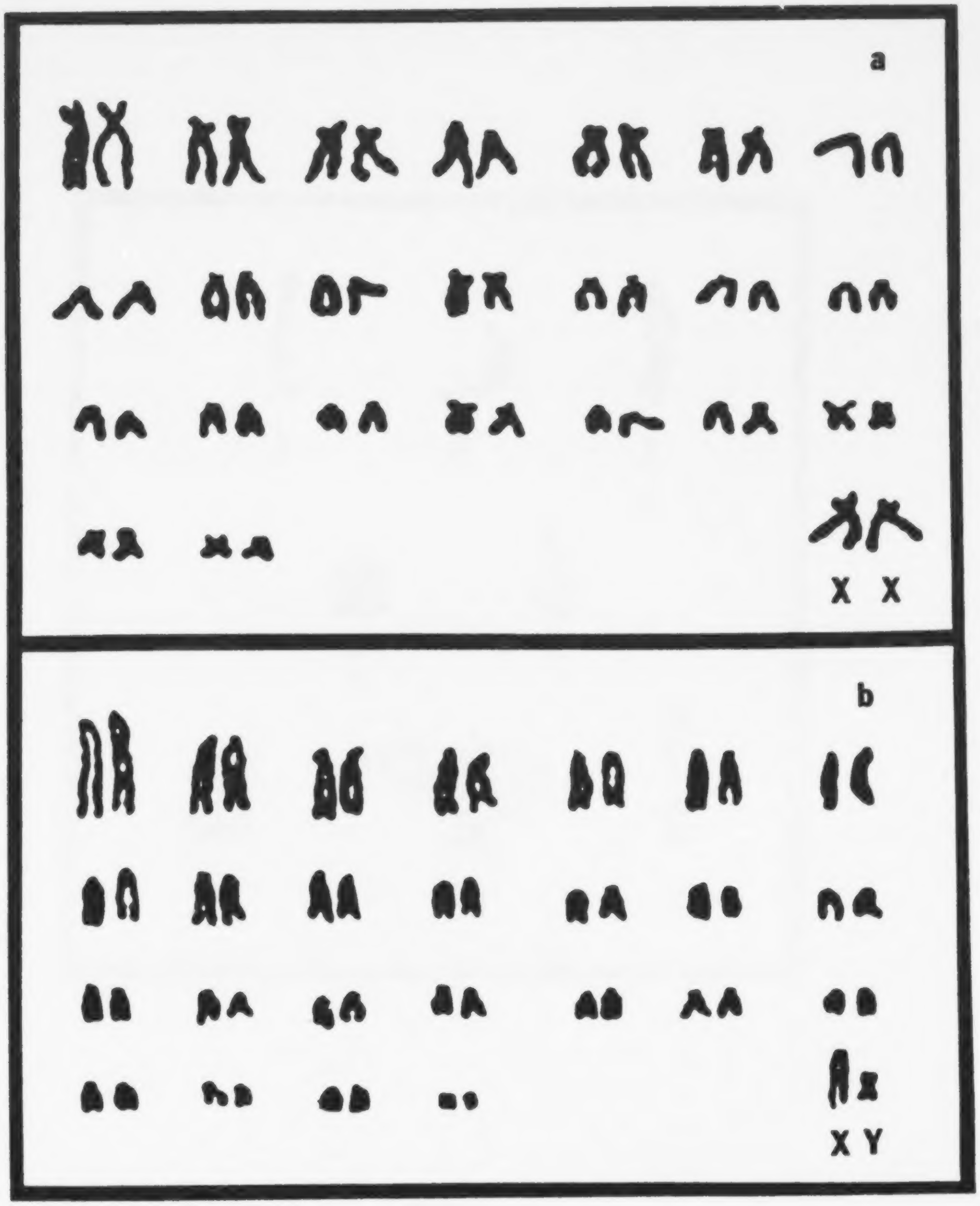

**Figure 9-8.** Normal geimsa stained standard karyotypes of a. Peromyscus leucopus, female, 2n = 48; b. Sigmodon hispidus, male, 2n = 52 (from McBee et al., 1987).

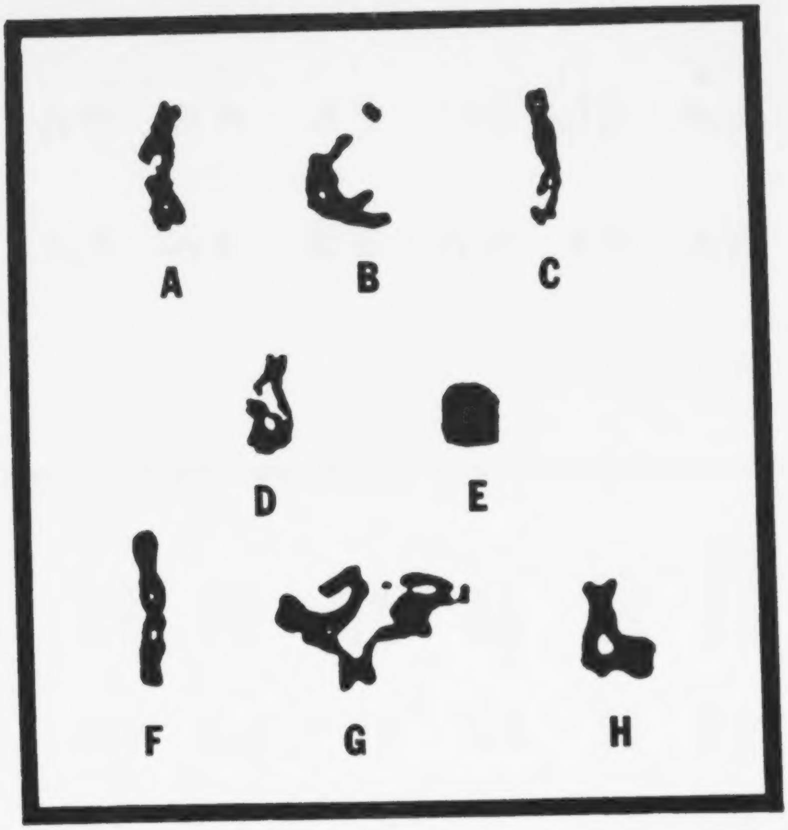

**Figure 9-9.** Representative chromosomal aberrations detected in standard metaphase chromosomal preparations of Peromyscus leucopus and Sigmodon hispidus from one field site (FS) and two control sites (CS1 and CS2). A-C. chromatid breaks, D. chromatid ring, E. chromosome ring, F. dicentric chromosome, G-H. multiple chromosome translocation figures (from McBee et. al., 1987).

## 9.5 REFERENCES

Akima, H. 1978. A method of bivariate interpolation and smooth surface fitting for irregularly spaced data points. Algorithm 526, ACM Transactions on Mathematical Software. 4:148-159.

Anscombe, F.J. Graphics in Statistical Analysis. American Statistician. 27:17-21.

Birge, W.J., J.A. Black, T.M. Short, and A.G. Westerman. 1989. A comparative ecological and toxicological investigation of an STP effluent and its receiving stream. Environ. Toxicol. Chem. In Press.

Brodlie, K.W., ed. 1980. Mathematical Methods in Computer Graphics and Design. Academic Press, New York, NY.

Carr, D.B., and W.L. Nicholsen. 1984. Graphical interaction tools for multiple 2- and 3-dimensional scatterplots. Pages 748-752. In: Computer Graphics '84: Proceedings of the 5th Annual Conference and Exposition of the National Computer Graphics Association, Inc., Vol. 2, May 1984, Anaheim, CA.

Carr, D.B., W.L. Nicholsen, R.J. Littlefield, and D.L. Hall. 1986. Interactive color display methods for multivariate data. Pages 215-249. In: E.J. Wegman and D.J. DePriest, eds. Statistical Image Processing and Graphics. Marcel Dekker, Inc., New York, NY.

Chambers, J.M., W.S. Cleveland, B. Kleiner, and P.A. Tukey. 1983. Graphical Methods For Data Analysis. Duxbury Press, Boston, MA.

Chernoff, D. 1973. Using faces to represent points in K-dimensional space graphically. JASA, 68:361-368.

Clark, I. 1979. Practical Geostatistics. Applied Science, London, England.

Cleveland, W.S. 1979. Robust locally weighted regression and smoothing scatter plots. Journal of the American Statistical Association. 74:829-836.

Cleveland, W.S., and R. McGill. 1984. The many faces of a scatterplot. Journal of the American Statistical Association. 79:807-822.

David, M. 1977. Geostatistical Core Reserve Estimation. Elesvrer Scientific Publishing Co., Amsterdam, Holland.

Davis, M.W. and P.G. Culhane. 1984. Contouring very large data sets using Kriging. Pages 599-620. In: Raidel, D., ed. Geostatistics for Natural Resource Characterization, Part II, Dordrecht, Holland.

Efron, B. 1981. Nonparametric estimates of standard error: The jackknife, the bootstrap, and other resampling methods. Biomtrika.

Efron, B., and G. Gong. 1983. A leisurely look at the the bootstrap, the jackknife, and cross-validation. The American Statictician. 37:36-48.

Environmental Systems Research Institute. 1987a. ARC/INFO Users Manual, Version 3.2. Environmental Systems Research Institute, Inc., Redlands, CA.

Environmental Systems Research Institute. 1987b. ARC/INFO TIN Users Manual, Version 3.2. Environmental Systems Research Institute, Inc., Redlands, CA.

Fienberg, S.E. 1979. Graphical methods in statistics. American Statistician. 33:165-178.

Hollander, M. and D.A. Wolfe. 1973. Nonparametric Statistical Methods. John Wiley and Sons, New York, NY.

Journel, A.G., and C.J. Huijbregts. 1978. Mining Geostatistics. Academic Press, New York, NY.

Linthurst, R.A., D.H. Landers, J. Eilers, and D.F. Brakke, eds. 1986. Chemical Characteristics of Lake Populations in the Eastern United States. Vol. 1: Population Characteristics and Physico-chemical Properties. U.S. Environmental Protection Agency, Washington, DC.

McBee, K., J.W. Bickham, K.W. Brown, and K.C. Donnelly. 1987. Chromosomal aberrations in native small mammals (Peromyscus leucopus and Sigmodon hispidus) at a petrochemical waste disposal site: I. Standard Karyology. Arch. Environ. Contam. Toxicol. 16:681- 688.

McLain, D.H. 1976. Two dimensional interpolation. The Computer Journal. 19:178-181.

Miller, W.E., S.A. Peterson, J.C. Greene, and C.A. Callahan. 1985. Comparative toxicology of laboratory organisms for assessing hazardous waste sites. Journ. Environ. Qual. 14:569-574. Ripley, B.D. 1981. Spatial Statistics. John Wiley & Sons. New York, NY.

Norberg-King, T. and D.I. Mount. 1986. Validity of effluent and ambient toxicity tests for predicting biological impact, Skeleton Creek, Enid, Oklahoma. EPA/600/30-85/044. U.S. Environmental Protection Agency.

Rochelle, B.R., D.L. Stevens, and M.R. Chruch. 1988. Uncertainty analysis of runoff estimates from a runoff countour map. Water Resources Bulletin. In Press.

Rowley, M.H., et al. 1983. Use of small mammals (voles) to assess a hazardous waste site at Love Canal, Niagara Falls, New York. Arch. Environ. Contam. Toxicol. 121:383-397.

Sibson, R. 1980. The Dirichlet tesselation as an aid in data analysis. The Scandinavian Journal of Statistics. 7:14-20.

Snedecor, G.W., and W.G. Cochran. 1967. Statistical Methods. The Iowa State University Press, Ames, IA.

Thiessen, A.H. 1911. Precipitation averages for large areas. Monthly Weather Review. 39:1082-1084.

Thomas, J.M., J.R. Skalski, J.F. Cline, M.C. McShane, W.E. Miller. S.A. Peterson, C.A. Callahan, and J.C. Green. 1986. Characterization of chemical site contamination and determination of its extent using bioassays. Environ. Toxicol. Chem. 5:487-501.

Tufte, E. 1983. The Visual Display of Quantitative Information. Graphics Press, Cheshire, CT.

# APPENDIX A

## ECOLOGICAL ASSESSMENTS of HAZARDOUS WASTE SITES WORKSHOP PARTICIPANTS

Elmer Akin
U.S. Environmental Protection Agency
Region 4
345 Courtland Avenue
Atlanta, GA 30365

Joan Baker
Kilkelly Environmental Associates
P.O. Box 31265
Raleigh, NC 27622
Federal Express: Highway 70 West
The Water Garden
Raleigh, NC 27612

John Barich
U.S. Environmental Protection Agency
Hazardous Waste Division
1200 Sixth Street
Seattle, WA 98101

Lee Barkley
U.S. Fish and Wildlife
Division of Environmental Contaminants
1000 N. Glebe Road, Room 601
Arlington, VA 22201

John Bascietto
U.S. Environmental Protection Agency
WH-527
401 M Street
Washington, DC 20460

Wes Birge
School of Biological Sciences
University of Kentucky
Lexington, KY 40506

Gabriel Bitton
Department of Environmental Engineering
University of Florida
Gainesville, FL 32611
904/392-0838
(will not attend the Seattle Workshop)

# APPENDIX A

# APPENDIX 1 (cont.)

Jerry Bromenshenk
University of Montana
Department of Zoology
Missoula, MT 59812
406/243-5648

Dave Charters
U.S. Environmental Protection Agency
Environmental Response Branch
MS-101
Woodbridge Avenue
Edison, NJ 08837

Patricia Cirone
U.S. Environmental Protection Agency
Environmental Studies Division
1200 Sixth Avenue
Seattle, WA 98101

Ken Dickson
Institute of Applied Sciences
P.O. Box 13078
North Texas State University
NT Station
Denton, TX 76203
Federal Express: Corner of Avenue B and Mulberry
General Academic Building
Room 471
Denton, TX 76203

Rich DiGiulio
School of Forestry & Environmental Studies
Duke University
Durham, NC 27706
919/684-6090

Michael Dover
375 Concord Drive
The Cadmus Group
Bellmont, MA 02178

Barney Dutka
Environment Canada
Canada Center for Inland Waters
867 Lakeshore Road
Burlington, Ontario L7R 4A6
416/336-4923

## APPENDIX 1 (cont.)

John Emlen
National Fisheries Research Center
U.S. Fish & Wildlife
Building 204
Naval Support Facility
Seattle, WA 98115

James Fairchild
National Fisheries Research Center
Route #1
Columbia, MI 65201
314/875-5399

John Fletcher
University of Oklahoma
Department of Botany & Microbiology
770 Van Vleet Street
Norman, OK 73019

Jeff Giddings
Springborn Life Sciences
Environmental Toxicology and Chemistry Division
790 Main Street
Wareham, MA 02571

Jeff Hatfield
Patuxent Wildlife Research Center
U.S. Fish & Wildlife
Laurel, MA 20708

Charles Hendricks
U.S. Environmental Protection Agency
200 S.W. 35th Street
Corvallis, OR 97333
503/757-4582

Kathryn Higley
Battelle
Pacific Northwest Laboratories
P.O. Box 999
Richland, WA 99352

Harvey Holm
College Station Road
U.S. EPA
Athens, GA 30613

## APPENDIX 1 (cont.)

Bob Hughes
U.S. Environmental Protection Agency
CERL/NSI
200 SW 35th Street
Corvallis, OR 97330

Elaine Ingham
Department of Botany & Plant Pathology
Oregon State University
Corvallis, OR 97331

Larry Kapustka
U.S. Environmental Protection Agency
200 S.W. 35th Street
Corvallis, OR 97333
503/757-4606

Hal Kibby
U.S. Environmental Protection Agency
200 SW 35th Street
Corvallis, OR 97333

Tom LaPoint
National Fisheries Research Center
Route #1
Columbia, MI 65201
314/875-5399
(will not attend the Seattle Workshop)

Will Laveille
U.S. Environmental Protection Agency
OEPER (RD 682)
401 M Street, SW
Washington, DC 20460

Greg Linder
CERL/NSI
200 S.W. 35th Street
Corvallis, OR 97333
503/757-4639

Karen McBee
Department of Zoology
LSW 430
Oklahoma State University
Stillwater, OK 74078
405/624-5555

## APPENDIX 1 (cont.)

Pat Mundy
U.S. Environmental Protection Agency
WH548A
401 M Street, SW
Washington, DC 20460

Ossi Meyn
U.S. Environmental Protection Agency
WH562B
401 M Street, SW
Washington, DC 20460

Susan Norton
U.S. Environmental Protection Agency
Office of Health and Environmental Assessment
RD-682
401 M Street, SW
Washington, DC 20460

Benjamin Parkhurst
Western Aquatics, Inc.
P.O. Box 546
Laramie, WY 82070
Federal Express: 203 Grand Avenue
Laramie, WY 82070
307/742-7624

Ron Preston
Environmental Services Division
U.S. Environmental Protection Agency
303 Methodist
Wheeling, WV 26003

Ron Stanley
U.S. Environmental Protection Agency
PM 223
401 M Street
Washington, DC 20460

Don Stevens
Eastern Oregon State College
LeGrande, OR 97850
503/963-2171

## APPENDIX 1 (cont.)

Glenn Suter
Oak Ridge National Laboratory
P.O. Box 2008
Building 1505
Mail Stop 038
Oak Ridge, TN 37831-6038
Federal Express: Bethel Valley Road
Oak Ridge, TN 37831-6038
615/574-7306

John Thomas
Battelle Pacific Northwest Laboratories
P.O. Box 999
Richland, WA 99352

William Warren-Hicks
Kilkelly Environmental Associates
P.O. Box 31265
Raleigh, NC 27622
Federal Express: Highway 70 West
The Water Garden
Raleigh, NC 27612

Ron Wilhelm
U.S. EPA
WH-527
401M Street S.W.
Washington, D.C. 20460

Bill Williams
U.S. EPA
200 SW 35th Street
Corvallis, OR 97330

Craig Zamuda
U.S. Environmental Protection Agency
Hazardous Waste Branch
PM220
401 M Street, SW
Washington, DC 20460

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